

nature

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Dependence or autonomy: UNCSTD's hidden agenda

With science-based technology playing an increasingly dominant role in the world economy, the politicisation of next week's conference has been inevitable. The major task is to make it constructive.

WHAT hope for UNCSTD? Delegates currently heading for Vienna, where the United Nations Conference on Science and Technology for Development opens on Monday, carry with them a fat set of gloomy prognostications. A successful conference — and one of the difficulties here is that a single criterion of success is almost impossible to define — still hangs in the balance. One thing is certain, however: not only is UNCSTD the last and possibly the most important of the major UN conferences to have taken place over the past decade — it has also proved to have been one of the most controversial.

There are numerous reasons for the disputes that have frequently threatened to engulf the conference preparations. Firstly there has been the growing recognition that the task of applying science and technology to development is in practice vastly more complex than it was thought to be a decade ago, when theories of technological determinism still reigned supreme in development policy. Secondly, it has become clear that many of the obstacles to this process — such as problems in energy or resource supply, or growing structural unemployment — have been partly due to the way that science and technology have been inappropriately applied in the past. Finally, perhaps less globally significant but important in the international context, has been the extent to which the issues faced by UNCSTD impinge on the existing interests and policies of other UN agencies.

Behind all these, however, is a factor which has received considerably less attention, but which seems destined to play a no less significant — perhaps even a dominant — role in the Vienna negotiations. This is the fact that over the past decade, the global economy has come to depend increasingly on science-based technology as the prime generator of wealth. And that consequently, for both developed and developing countries alike, issues relating to the ownership and control of technology have become as important in a political sense as the use of this technology has become in economic terms.

One result is that for the developed countries the whole issue of science and technology for development has increasingly shifted from the sphere of aid to that of foreign policy. As the developing countries have clamoured for political autonomy, so the interdependence between nations has shifted from the militarily-backed relationships of colonial times to the economic domain. The desire for technological know-how, as well as the conditions under which this is created, bought and sold, have subsequently moved to the centre of these interdependent relationships. In addition, political issues surrounding the impact of technology on society have increasingly taken on an international dimension.

Nowhere have the implications of these developments been more clearly recognised than in the United States. One of the most significant recent trends in foreign policy strategy has been a shift from the aggressive military stance that became so discredited during the Vietnam era, to a style of soft-shell diplomacy emphasising economic cooperation and mutual self-interest.

Science and technology, through cooperative agreements, collaborative research ventures, and academic exchanges, have become a central tool in this new approach. Dr Henry Kissinger was one of the first to point out that problems such as energy, resources, and the uses of space and the sea ranked with questions of military security, ideology and territorial rivalry which had traditionally made up the diplomatic agenda.

The use of science and technology as a tool of diplomacy has received even greater support from the Carter administration. On the one hand it has resonated with the President's own engineering perspective on social problem-solving; on the other, it has been actively supported by the US business community as an alternative to more overtly coercive forms of global involvement. Perhaps the most obvious success of this new strategy has been the use of scientific and technical agreement to finesse previously stalled diplomatic initiatives with the People's Republic of China, a possibility opened up by the new regimes desire for access for US technology. Since then, it has been used extensively to articulate relationships with both developed (e.g. West Germany and Japan), and developing countries (particularly Mexico and Latin America), and the approach has received substantial backing from the US Congress.

To this development must be added another which has also emerged since the deliberations for UNCSTD first began, namely the developing countries' demand for the establishment of a New International Economic Order (NIEO). Both have since come to dominate the perspective from which the developed and the developing countries respectively are approaching the Vienna negotiations. And where conflicts and disagreements have arisen in the preparatory process, many can be traced back to the tension between these two goals. For what the US sees as the use of science and technology in, to use one of Dr Brzezinski's favourite phrases, "the stable management of global change", developing countries see as an attempt to reinforce US hegemony in a new guise. And the latter have also concluded that issues concerning the control of science-based technologies are a key to the restructuring of international relationships envisaged in the NIEO.

Delegates meeting in Vienna next week are not substantially divided on what needs to be done, whether at the level of meeting human needs for adequate food, energy and housing, of increasing research efforts on issues relevant to these and other developing country concerns, or of building up research capacities in the LDCs. The main source of dispute is over *how* these goals should be tackled. And here the central arguments share many of the characteristics of the debates that have taken place within the developed countries over the past few years on the relationship between science and society. Given the problems that have accompanied society's use of science in the past — the pollution of the environment, the heavy imbalance towards military research and technology, the alienation of "scientifically managed" labour and processes, and so on — can better solutions



be found by revising the responsibilities of existing institutions? Or are the problems endemic to these institutions, and does their solution require, as radical critics would suggest, a more fundamental restructuring of social and political relationships?

Transposed to the international arena by the UNCSTD conference, the answers being given to these questions seem clear — and frequently in conflict. The developed countries, while not minimising the difficulty of applying science and technology to development, argue that this can still be done most effectively within the existing pattern of international relations. The message they bring to Vienna is that the task requires the appropriate mix of pragmatism and private enterprise with the former intended primarily to fill the holes left by the latter. As one group of US businessmen put it in a report to the States Department, a prime need is “to support and expand the existing system which on the whole has been the source of an unprecedented advance in science, technology and economic development throughout the world.”

The developing countries, at least as far as their views have been presented in the preparatory process by the Group of 77 — the “trade union of the poor” according to President Nyerere of Tanzania — see the situation differently. They argue that the political structures which support the global economy are biased in favour of the rich, industrialised nations, and that a more equitable distribution of bargaining power is central to the NIEO. In the UNCSTD context this means not only building up their own research capacities, but easier (and cheaper) access to the technology already existing in the developed world, and a substantially greater share of control over the global research and development budget.

The developed countries have, not surprisingly, dismissed such demands as “unrealistic”. For example, a central objection to the international financing system which the Group of 77 has proposed to support science and technology activities has been the political unacceptability of the developing countries laying down how much the developed countries should contribute to this fund; the latter would prefer to operate through, for example, the United Nations Development Programme, where they can exercise greater control over the size and use of their contributions. In addition, developed countries argue, with some justification, that there is little domestic support for increased aid spending at a time when foreign competition is blamed as one reason for economic recession. Nor is their much enthusiasm for the political goals of the NIEO, whose provisions, according to one US industrialist, “will be a discouraging factor affecting the final resolutions and resulting programming” of the UNCSTD meeting.

In return, there exists a deep distrust among many developing countries about the way that the developed countries conceive the task of applying science and technology to development. The predominant fear is that the types of solution offered, while dressed up as cooperative ventures, will in practice be used as Trojan horses to reproduce precisely those dependency relationships which, they feel, must be broken if a just and equitable global development is to be achieved. Internationally they feel that the continued hegemony of the industrialised nations on research matters implies a dependency which only sanctions patterns of development meeting such nations’ interests. Domestically there is concern — voiced more frequently from unofficial rather than official quarters — that solutions designed primarily for their compatibility with a global market economy will favour local elites already integrated into this economy, at the expense of the poor who remain marginal to it (apart from providing a source of low-cost labour power). Thus in both cases it is feared that unless sufficient endogenous control is exercised over research and development programmes, however scientifically and technologically appropriate their goals, their benefits will tend to be skewed towards the relatively rich and powerful, and against the poorest and those most in need.

Given these conflicting perspectives, it is perhaps inevitable that the issue of control has become central to virtually all current debates where science intersects with development concerns —

what might be called the field of development science policy.

As far as global research projects are concerned, it is already clear that one man’s pragmatism is another man’s political programme. Issues such as exploring the mineral potential of the sea-bed or attempting to prevent the proliferation of nuclear weapons have become highly charged as developing countries see in proposed procedures attempts to restrict their political rights. On the transfer of technology, the broad parameters of the conflict are already widely known, with the developed countries arguing that technological know-how be respected as the private property of the inventor, and the developing countries preferring to see technology more as part of the common heritage of mankind. Even in the debate over the need for appropriate technologies, developing country scepticism has focussed less on the technologies themselves than on the motives of those who suggest their widespread dissemination.

In sum, what is being challenged at UNCSTD is the extension of the institutional framework and political assumptions which have supported the growth of Western science and technology — the so-called social relations of science — as the appropriate model for applying science to society’s needs on a global basis. To the extent that these social relations of science have served the interests of the Western market economies, as well as a number of their socialist counterparts, those who see a global economy operating along the same lines argue that they are appropriate to the wider context. But to the extent that they are seen as presenting obstacles to genuine development, creating unwelcome dependencies, reinforcing inequalities in wealth and power, and condemning those most in need to the economic margins, these social relations will be challenged by critics demanding a far greater control from the grass-roots of the processes leading to both the generation and the application of scientific knowledge.

Where, then, does this leave our hopes for UNCSTD? Putting political realities aside, we could envisage a set of decisions that might include: agreement substantially to increase the proportion of the global R & D budget devoted to development-related topics, say from 5 to 25 per cent by the year 2000 (in line with the Lima target on world trade); agreement to halve the proportion of this budget spent on military research by the same date; agreement that restraints on proprietary technology be substantially reduced where these present a major obstacle to their global deployment; and so on.

Pipedreams and magic wands, however, will have little place in Vienna. At present it would be wrong to expect any quantum leaps in development strategies to emerge (even though these will be hotly supported in the NGO forum). At best we can hope for some agreement on the type of actions that developing countries should take to build up their scientific and technological infrastructures; a limited commitment from the developed countries to reallocate some of their aid funds to R & D objectives; a new focus within the UN system to remind all countries of the importance of science and technology in development strategies; and perhaps preliminary agreement to extend current efforts in fields such as information dissemination and remote sensing.

This may sound pitifully little for an exercise which has taken up so much time and energy over the past few years. But the real gains may be less tangible. The conference secretariat constantly reminds us that, for many countries, merely preparing a position paper for the conference has been a highly instructional exercise. In the same way the conference proceedings themselves may highlight one topic which has been dropped from the explicit agenda, namely the obstacles that obstruct the applications of science and technology to development. (For example through clarifying major areas of disagreement, and analysing the reasons). If no more, delegates will be exposed to the challenge and the difficulties of developing a common frame of international discourse embracing political and technical issues simultaneously. This in itself will do little to change the world; but it is a prerequisite for the issues that need to be negotiated in the decades ahead. □

UNCSTD: what's to be done?

The UN Conference on Science and Technology for Development starts in Vienna next week.

We asked a group of scientists, politicians and administrators to pinpoint the most critical action that needs to be taken

The proper place for science in the Third World

by Joao Frank da Costa
Secretary-General of UNCSTD

I AM frequently asked "how are you going to assess if the Conference on Science and Technology for Development is a success or a failure?" It is not an easy question to answer. Obviously, the difficulty in reaching agreement on conference issues should not be the sole criterion. I believe incidentally that there are only three main issues: 'transfer of technology' — which evokes emotional overtones on both sides; the institutional mechanisms for science and technology in the UN system; and, above all, the financial arrangements. They are extremely difficult problems that have been discussed or negotiated without success for decades, and there is no objective reason why, in these times of extreme economic difficulties, dramatic breakthroughs should be considered possible.

Another criterion could be the ability and willingness to arrive at a common agreement on the text in the Draft Programme of Action. For my part, I would have liked to see a Programme of Action containing fewer brackets than the text which resulted from the fifth session of the conference preparatory committee (PrepCom), so that the conference could concentrate on only a few critical issues. The time-table for the PrepCom was however too short, and the exercise undertaken was not much more than a first reading. In addition, it seems to me that the negotiators considered the text as a kind of treaty and not, as they should have, as a general recommendation directed to member states, international organisations, and the UN system; and as a recommendation that still needs to be channelled and endorsed by proper mechanisms like the General Assembly.



In our case, neither of the criteria seem to be entirely valid. I believe that the conference will have achieved at least part of its purpose if it results in the recognition of the specificity and the importance of science and technology as tools for development, and their incorporation into national planning and international programming at all levels. I consider that this purpose has already been largely achieved at the national level. An enormous number of countries (and not only the developing ones) have studied, in the context of the preparations for the conference, the difficult problems involved in the application of science and technology for development. They have also achieved a measure of coordination internally, not only between the different government departments concerned but also between producers of science and technology (mainly the scientific and technological community) and its users (the public in general). Institutional arrangements have been initiated or established, and rudimentary structures have been replaced by more comprehensive and modulated mechanisms for development, at both the national and the supranational level.

The success of the conference could also be judged by the political will shown by member states in providing the tools required to monitor the results of the conference — whether these are programmes of action, declarations or resolutions — and the necessary financial means needed to strengthen the endogenous capacities of developing countries. It is obvious that an international high-level mechanism like an intergovernmental body is needed to monitor the implementation of the results of the conference — otherwise, these would remain on paper. Similarly, the strengthening of the endogenous capacities of developing countries would need some new international financial arrangements, and in the absence of these arrangements, it will remain a pious platitude.

Of course success will only be complete if the specificity and importance of science and technology for development are also recognised at the international level. I

believe the second part of the conference (the Vienna conference itself) should be assessed by the importance accorded to those two elements.

The conference is not an end in itself. It is both a reflection on what has been done in the past (rather little I am afraid) and what needs to be done in order to provide each country with a capacity to solve its own problems and to create an international environment conducive to such an endeavour. And it should, of course, also be considered against the background of the New International Economic Order and in the context of the formulation of a new International Development Strategy.

Strengthening the scientific infrastructure

Michael J. Moravcsik

AMONG the various guidelines suggested for UNCSTD the one with perhaps the broadest worldwide support urges that UNCSTD should result in concrete steps to strengthen the scientific and technological infrastructure of the developing countries. Such an aim has a number of advantages:

- it touches on the crux of the matter and is not just a palliative;
- it is relatively low-profiled politically, since many who are reluctant to share specific knowledge or resources are nevertheless willing to share the capability of generating knowledge;
- it is relatively inexpensive and does not require large new lump sum expenditures;
- it can be implemented in a decentralised fashion, thus avoiding the creation of new international or national bureaucracies;
- it brings partial benefits if it is partially implemented, in contrast to grandiose, all-or-nothing schemes.

There are many problems in infrastructure building, and UNCSTD can address only a few of them — it does not matter much exactly which. Science and

technology education of specialists and of the public, inside and outside with technology, and technology with production; and scientific research and technological development: these are all areas in which many specific, realistic, and effective proposals await implementation. Such programmes must involve the direct participation of working scientists and technologists the world over.

The most important aim is to ensure that UNCSTD, unlike the long list of other UN conferences, produces something tangible and operationally useful, even if only on a modest scale.

Dr Moravcsik is at the Institute of Theoretical Science, University of Oregon

A ten-year ban on large and poorly prepared meetings

Ward Morehouse

Now that the final UNCSTD Preparatory Committee meeting is over, it is possible to predict with a modest measure of certainty the likely outcome of the conference — a programme of action filled with platitudinous recommendations for national governments, especially in developing countries, to do more for and with science and technology for development; a new high-level committee and some rearrangement of concerned secretariats at the UN; a charge to the UN Development Programme and the specialised agencies to pay more attention to science and technology in their activities; and possibly some very marginal increase in the resources available from development agencies, mostly by reallocating existing budgets.

In the meantime, the cost of UNCSTD is approaching \$50 million — not just the direct UN secretariat and conference costs (\$7.8 million) but also the lodging, food and international travel expenses of the 4,000 people expected in Vienna during the last two weeks of August (\$6 million), the professional compensation of the 2,500 government delegates and UN officials preparing for and attending the conference (\$3.8 million), the host country's budgetary allocation for UNCSTD (\$5 million), the five PrepCom meetings (\$3.2 million), the preparation of national papers and related preparatory activities of 100 governments and an assortment of UN and other international agencies (\$14.6 million) and the 100 or more international conferences and seminars spawned by UNCSTD (\$8 million).

The likely outcome of UNCSTD seems marginal in relation to such expenditures. Even if a four-part package involving a \$200 million science and technology fund, which is now being quietly negotiated, were to happen (an outside chance), it is not

clear whether the result would do more to benefit scientists in North and South who say they are concerned with development or to improve the lives of poor people by bringing them the benefits of science and technology — through, as the principal UNCSTD discussion paper expresses one of the major goals of the conference, "the establishing of a just and equitable social order."

In fact, the real danger of exercises like UNCSTD is that they divert attention from the underlying economic, social, and political causes of human suffering and deprivation by deluding us into thinking these problems can be solved by science and technology alone.

Hence I propose, as one important action to enhance the realisation of the UNCSTD goal expressed above, a ten-year moratorium on large and indifferently prepared international meetings on science and technology for development, and similar global problem-solving conferences, while we search for more cost-effective ways to link the North-South dialogue to meaningful efforts to ameliorate the human condition. The money thus saved can be used in the coming decade to get on with the task of improving poor peoples' lives by whatever means are most appropriate, including — but not limited to — science and technology.

Dr Morehouse is President of the Council on International and Public Affairs, New York

Radical changes in UN procedures and policies

A.B. Zahlan

THE deliberations that underlie UNCSTD revolve around three particular problem areas. North-South relations, internal transformations of developing countries and the UN system as an instrument of technical assistance. Although all three require attention, it is perhaps fitting that UNCSTD should be primarily concerned with how the performance of the UN system could be improved. The UN system is not merely a source of aid; it also diffuses procedures and models that are often copied by the institutions of the Third World. The subject matter concerned with each of these problem areas is extremely complex; the extent of awareness of the specifics of the processes is poor; and the vested interests in the bureaucracies of UN agencies, ministries of Third World countries, the business communities and the governments of industrial countries are such that a stalemate has been attained. This situation is not in the interest of either the world community or the UN system; and it is certainly not in the interest of the Third World.

The efforts so far deployed to effect the

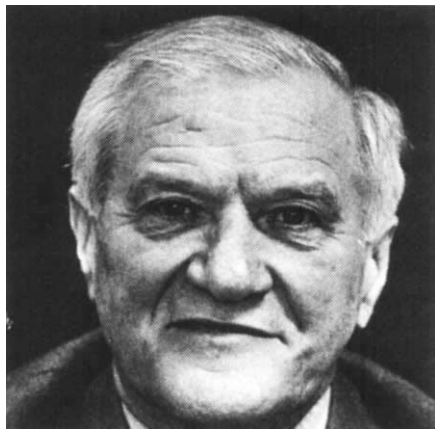


Da Costa: UNCSTD Secretary-General

changes called for by the New International Economic Order and by UNCSTD are too limited to dislodge established practices. The tens of thousands of persons employed in this multi-billion dollar 'aid industry' cannot spontaneously switch off past practices and become pioneers of the New International Economic Order and UNCSTD. The 'aid industry' operates on the basis of concepts of development that originate in both advanced and developing countries, and all these parties must share in altering accepted dogma. The management of the UN system has often attracted attention, but so far the system has not been opened up to provide factual data on its operations, performance and the policy implications of its practices. Yet the technological development of the Third World, along the lines proposed by UNCSTD, calls for radical changes in the procedures and policies underlying the operations of UN agencies. In theory the UN system is subject to scrutiny by member states at the level of the General Assembly, and during the general conferences of the respective agencies. In fact, however, very little of the necessary factual examination and analysis actually takes place at any of these gatherings. Short-term interests dominate, and few of the delegates possess the adequate information to assess the facts. Currently these international discussions are remote from the scientists, institutions and problems of the Third World. It is only through a dedicated concern about the field aspects of science and technology that the debate can be enriched with facts and realism.

The challenge facing the Third World in the UN system today is not to win a vote or to issue a new proclamation, but rather to initiate a process powerful enough to transform the practices of the system. A revitalised UN system has a good chance of accelerating the mobilisation of the internal resources of developing countries. These resources are formidable and all too often underestimated.

Prof Zahlan has been a scientific advisor to the UN (on ACAST) and Professor of Physics at the American University, Beirut. He is presently a visiting fellow at the Science Policy Research Unit, University of Sussex



Dedijer: information revolution

A basic capacity to absorb and create knowledge

Hiroshi Harada

THERE is much that can be said on the topic of 'Science and Technology for Development', but as far as developing countries are concerned it is useless unless they have in the first place a basic capacity to absorb and create scientific and technological knowledge, and to be adept in its use for productive and commercial purposes. This obvious fact seems, nevertheless, to be often overlooked, as is the fact that there is no short cut to acquiring it.

Education is the only universal means for forming this capacity from the innate intelligence available. And in this context, education must not be regarded solely as a process of passing on information and experience, but one which stimulates the curiosity of its recipient and develops a sense of applicability of the knowledge acquired. It must increase practical ability and the powers of observation and communication. It must instil mental self-discipline and ruthlessly maintain standards and integrity in study and research. These — and a willingness to be self-critical — are essential for self-reliance, both individually and nationally.

The process must be started deliberately at the primary level of education. It is too late at the secondary and higher levels, when the sheer weight of knowledge to be acquired can inhibit any burgeoning sense of mastery over it. This applies whether the product of the educational system is a professional scientist or technologist, or a layman. Indeed, an appreciation by the latter of what is involved is essential if the impact of science is to be accepted and the technological revolution necessary for development is to take place. Otherwise, scientists and technologists become, as is seen too frequently in developing countries, elitist and largely ineffectual in the development process.

Prof Harada is a Japanese plant physiologist, currently with UNESCO

Common sense, perseverance and ingenuity

John Ziman

IN the short run, the developing countries are seeking scientific and technological information; in the long run, to make use of that information and to serve their own needs, they will need research capability.

● In the short run, that means material facilities; in the long run, these are merely the instruments of scientific institutions.

● In the short run, a scientific institution is a bureaucratic organisation; in the long run it must grow into a scientific community.

● In the short run, a scientific community is made up of technically qualified personnel; in the long run, it depends on the leadership of its scientists.

● In the short run, a scientist is an expert who has passed the examinations for a PhD; in the long run, this is only an apprenticeship to the profession of research.

● In the short run, the goal of research is to make specific contributions to knowledge; in the long run, it is to exercise curiosity and to solve problems.

● In the short run, problems are often solved by the application of sophisticated, specialised expertise; in the long run this calls for common sense, perseverance, and open mind and ingenuity.

A scientific institution with some capability for research can be set up in about 5 years. Scientific workers with some research experience can be trained in about 10 years. It takes a generation or so of education and cultural change to foster the personal qualities needed to solve practical problems by scientific methods. This path is long and narrow, but it is the only known way. For the developing countries there is no time to lose: they, and we, must start now.

Prof Ziman is head of the Department of Physics, University of Bristol

Breaking the monopoly on scientific knowhow

S. Dedijer

I SEE three threats in UNCSTD to developing countries developing their own capability to find out, to discover, to invent and to innovate in both predictable and unforeseen circumstances. Two of them are strongly backed proposals for deceptive solutions to the problems which arise from the fact that almost all the global scientific and technological output, capability and knowhow is concentrated in only 30 countries, from which the

remaining 80% of the world has to import.

The first threat would arise if UNCSTD set up one more UN organisation to 'deal' with the problem, without a critical evaluation of the efficacy of the existing ones. The second threat could come from the search for solutions to the problem in political attacks against 'capitalist dependence' of the developing countries without the realisation that such non-'dependent' countries as the USSR and China are almost colonially dependent on the science and technology of the democratic countries.

The most serious threat is that the developing countries, as shown by their UNCSTD national papers, have done their homework badly. Not one of them seems to have yet discovered the very fact of the new electronic information revolution, or has realised its implications for development. This includes the fact that information democracy — the right and motivation of everyone in a country to obtain, produce, distribute and withhold information — is becoming essential for a country's ability to acquire and create scientific and other knowledge and knowhow. Democracy, in other words, is becoming a productive force.

UNCSTD itself offers the delegation from every developing country the opportunity to test critically the approach, the findings and recommendations in their national papers and to apply them creatively for national goals after UNCSTD.

Prof Dedijer is at the Research Policy Institute, University of Lund

Active partnership of Third World scientists

Yongyuth Yuthavong

THE challenge of science and technology today is not to show that it can give rise to social benefits, but that these benefits are produced where they are most wanted — in the Third World. There is an urgent need to direct a far larger part of world scientific and technological activities specifically towards Third World development. Solutions to many development problems have to be sought largely within the developing countries themselves, and their scientists and technologists must be given the necessary means to search for them. Solutions to many problems, for example in the fields of population, nutrition and health, are already known but lack implementation. An integrated approach, including all necessary social, economic and political components is needed.

The developed countries have the capacity to find scientific solutions to many basic problems concerning the survival of mankind, and should make more effort in this direction. However, ultimately, effective action in the Third World

depends on the competence and morale of its own scientists, technologists and other colleagues, and sufficient appreciation by its general public of the value of scientific solutions to development.

Support from developed countries could take the form not only of direct financial assistance, but of collaboration between individuals and agencies concerned with issues ranging from basic research to industrial consultation, and the dissemination of packaged technology to an illiterate rural population. In short, an active partnership of Third World scientists and technologists is an important prerequisite for success of development efforts. It is, furthermore, important to make the general public in the Third World — especially the few leaders with so much influence — aware of the critical role of science and technology in development, and to make the necessary tools available.

Dr Yuthavong is at Mahidol University, Bangkok

Three imperatives: food storage, energy, work ...

Guido Brunner

A GREAT responsibility rests on those who will be taking part in UNCSTD in Vienna. They have to make a fundamental choice. They will either be drawn into a discussion of abstract principles of a new international economic order, or — and this is what I hope to see — they will get down to some practical business. The first will merely lead to yet another diplomatic deadlock of the kind we have seen already too often in meetings between the developed and developing world. The other will spark off that real scientific and technical co-operation which is so necessary for the solution of the problems of development.

This is an especially significant time for the conference. Prospects for the world economy are clouded, and governments are worried about the rising cost, and shortages, of energy. For the developing

world the three imperatives are: the storage of food, energy and work. The conference must see that the proper scientific and technological resources are freed to contribute to these great tasks.

A long term effort internationally and at home will be required if the developing countries are to build up the necessary internal scientific and technological capability. The transfer of technologies from the developed to the underdeveloped is only one aspect of the problem. What is equally important is to establish the means for the developing countries to improve their educational and managerial standards.

The Community and its nine Member States will play their part in this great venture. Our association with 57 developing countries in Africa, Asia and the Caribbean within the Lomé Convention has already enabled us to make a contribution, and the new Lomé Convention will provide us with a framework for offering additional support for scientific and technical co-operation.

Dr Brunner is Commissioner of the European Communities for Energy, Research, Science, and Education

Policymaking should be at the highest level of authority

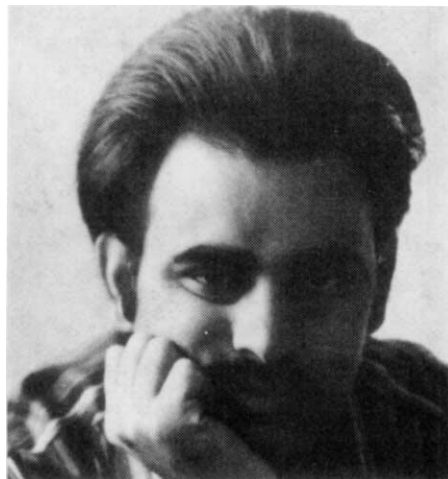
Antonio Pinilla

THE most pressing need is for a strong, deep and consistent political decision that science and technology constitute essential elements for each state to obtain:

- social integration;
- economic development;
- cultural independence;
- political coherence; and
- administrative efficiency and productivity.

Inadequate knowledge generates racial, social or religious prejudice, frustrating the national integration of countries. Economic growth requires agricultural and industrial mechanisation and rationalisation and these are impossible without a certain advanced degree of scientific and technological development. Payment of duties and royalties make national products uncompetitive in international markets. Economic dependence often generates cultural dependence, so decreasing awareness of national identity. Political and administrative sciences are key solutions to problems concerning political coherence, continuity, administrative efficiency and productivity within a free society.

For these reasons the making of science and technology policy should be the responsibility of the highest levels of authority, such as the president of the Republic (Head of State), parliament and



Sardar: self-confidence

the cabinet. The person in charge of implementing science and technology policy should have the rank of minister of state, or even better, be a full cabinet member.

The usual type of problem confronting each cabinet member is something very specific and urgent. Cabinet members cannot afford to concentrate on the scientific and technological aspects of the problems they face. That is why it is critically important that present-day states create national systems of science and technology, giving them the highest level of responsibility and authority to ensure scientific and technological aspects of national plans and objectives, policies and actions are sound, do not include errors or insufficient information, and are adequate and appropriate to each national reality.

Prof Antonio Pinilla is President of the Peruvian Consejo Nacional de Investigacion Cientifica, Lima

Inculcating an appropriate sense of confidence

Ziauddin Sardar

ALL developing starts with the individual, and science development is no exception. The first and the most critical action, in my book, concerns the scientists of the developing countries themselves: every Third World scientist must consciously inculcate in him/herself an appropriate sense of confidence. Science can only play its full role in development when the integrity of those who do science is preserved. And this integrity cannot be preserved when the Third World scientists themselves do not have any confidence in their ability to manage their enterprise.

There is a natural corollary to the development of confidence in Third World scientists: confidence in oneself precedes from confidence in one's society, tradition, culture and institution. If someone has confidence in his ability as a scientist and



Brunner: fundamental choices



Salam: 10 times more scientists

his culture and society, then he does not look down on the needs of the society as 'insignificant' and indigenous research as 'inferior'; nor does he apologise away his research efforts to solve local problems or place too heavy a reliance on outside help and consultants and advisors. Furthermore, a scientist with confidence in himself builds up confidence in his colleagues and does not feel threatened by younger scientists. Nor does he articulate the desire to block the paths to full realisation of those who have potentially more to contribute to science development.

As far as I am concerned, science development in the developing countries begins and ends with the confidence the Third World scientists have in themselves, their society and the science that they practise.

Dr Sardar is at the Hajj Research Centre, King Abdul Aziz University, Jeddah

Local scientific committees are too small

Abdus Salam

I HOPE the conference can focus on and initiate action on the following important area. Any declared intention of applying science and technology to development is — and will remain — meaningless until the developing countries build and deploy indigenous scientifically and technologically trained communities. At present, however, such communities simply do not exist, or their sizes are tenths or hundredths of what is needed. Their direction and deployment must be the concern of scientists and technologists themselves, and not of the economists and bureaucrats of national and international planning commissions.

It must be realised that whatever priorities the countries set themselves (food production, mineral exploitation, transport, health, manufacture or even

defence), a meaningful application of science and technology needs commitment; is not cheap in money, men or time; and it brooks of no magic formulae.

Turning to the reciprocal role of developed countries, we note that the theme of world development has been woefully neglected by the scientific and technological communities of the developed countries, who have made little organised effort to help in this task, nor have they collectively shown any great vision. The same applies to their state agencies.

In addition, the international funds at the disposal of bodies like ICSU — or even UNESCO — for the development of science and technology for global concerns are pitifully small. (The entire UNESCO budget — not to restrict it to what is spent on science and technology — is smaller than that of the Ford Foundation). These international funds need to be increased by at least an order of magnitude.

Prof Salam is Director of the International Centre for Theoretical Physics, Trieste

A large debt remains for past exploitation

Joseph Hanlon

THERE is a need for a 'new scientific order', like the new economic and information orders, which attempts to break the stranglehold of the developed over the developing nations. Scientists in developed nations must realise that the science gap has not come about by accident. During the past 200 years of scientific progress, most Third World nations were colonies which were not permitted to develop an independent scientific capability. Small wonder, then, that there is a gap now.

The challenge today, especially for UNCSTD, is that the developing nations are working hard to maintain and widen that gap. The brain drain continues, with the developed nations sucking the brightest scientists into their companies, health services, and universities. Patent laws are now being used to block Third World research into key areas such as alternative energy. Journals and scientific societies, controlled by developed world scientists, define 'good' science as that which is of interest to developed nations. Tied aid ensures that experts and equipment come from such a wide variety of countries that even the most talented local scientist cannot digest and control the results.

All of this ensures that in those scientific areas which are of most importance to the developing nations — health, energy, ecology and geology — the expertise and control will remain with the developed nations.

UNCSTD can help to end this exploitation. But it will require the realisation in the developing nations that they must stop bowing to the demands of journals and aid agencies in the industrialised world. And it will require that scientists and science policy makers of the developed world understand that a large debt remains to be paid for past exploitation, and that a way of repaying is to provide increased aid — not for what they think the Third World needs, but for what local scientists and policy makers feel is required.

Dr Hanlon is a development journalist

Loosen the grip on proprietary rights in technology

Jose Goldemberg

THE most critical actions necessary to improve science and technology in the developing world are probably beyond the powers of UNCSTD, or of the United Nations.

Science, especially technology, does not flourish in the Third World — not because of incompetence amongst the people involved (although that exists too) — but because it is not really needed in the present model of development being followed in most of the Third World. Machines, technical expertise and a powerful marketing system coming from abroad inundate the developing countries, ministering to some of the needs of the people, creating others and above all generating a craving for the comforts of modern civilisation as defined by the industrial nations.

There is little that science can do to interfere with this situation, because most of the science needed exists already, and there are no fundamental new discoveries that could change the situation or the climate in which science could flourish in the Third World.

Technology, however, has to be adapted, in many cases to local conditions (of temperature, humidity, and so on) and to local fuels and materials. Native technologies have a role to play there, but in general technology is imported.

The most critical action to take in my view would be to loosen the grip on proprietary rights in technology. If these were made available and essentially free, local industries would have more room to move and more scientists and technologists would be needed to make choices and develop missing links. One thing that could accelerate this process would be to have international funding institutions which preferentially support projects that use as much native technology as possible.

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Soviet aid: a two-way traffic

The eastern bloc's relations with the Third World stress 'real reciprocity'. Vera Rich reports

Science and technology transfer from the Soviet Union and other Comecon countries to the Third World is seen, not surprisingly, somewhat as an extension of such transfers within the bloc itself. That is to say, it is viewed not as "aid" — a word properly reserved in Soviet terminology for *ad hoc* measures such as the recent earthquake relief to Yugoslavia but as an essentially reciprocal process. (The official name of Comecon is the Council for Mutual Economic Assistance).

One interesting outcome of this approach is the attitude to "intermediate technology". The long-term plans of Comecon aim at integrating the economies of the member states, hence there is clearly a pressure to bring such countries as say, Hungary and Bulgaria, from a peasant-agrarian to an agro-industrial economy in the shortest possible time.

At least, this is the case in the production sector. The current Mongolian exhibition in London suggests that, although the Mongolian People's Republic boasts a state university, an academy of sciences, polytechnic, medical research institutes, a flourishing pharmaceutical industry and a major programme for the exploitation of mineral resources, a significant proportion of its population still lives, by choice, in the traditional *ger* or felt tent, though, on the outskirts of the capital, Ulan-Bator, gers are frequently fitted with such modern conveniences as electric light and television.

Regarding the third world, Soviet commentators stress that the bulk of what they offer is not the "relatively primitive mechanisms and implements which, in the opinion of some foreign theorists, are the most appropriate to the present requirements of the 'third world' countries", but "modern technology... which can contribute to the developing countries' mastering of modern scientific and technological achievements". Similarly, when co-operating with the developing countries, the Comecon countries have tended to follow current practice within the bloc as far as know-how is concerned.

As a result, the transfer of technological documents to the third world, originally free of all but incidental costs, has increasingly become replaced by the sale of know-how and licences (usually against long-term credits), and special trading organizations have been set up in the various Comecon countries to handle this. These include *Litsensintorg* for the Soviet Union, *PolSERVICE* for Poland, and *TESCO* for Hungary.

Hungary is a particularly interesting case of such co-operation, since, until recently, it was itself eligible for aid under the United

Nations Development programme. TESCO, the Technical and Scientific Co-operation Organisation, was founded in 1962, and at present operates co-operation agreements with 57 developing countries. One of the directors of TESCO, Mr Istvan Orosz, told *Nature* recently that Hungary cannot offer such assistance free, although sometimes it may be provided at basic costs. Another director, Mr Sandor Derzsi, stressed that in the case of countries which have recently been "liberated from colonialism" the main form of co-operation is the sending of Hungarian experts (in all, nearly 1000 annually), whose salaries are, if necessary, paid by the Hungarian government.

As the countries develop, he said, the form of co-operation can change. This was the case with Cuba, for example. Originally Hungarian experts went to Cuba, and Cuban trainees studied in Hungary. "But now we have bilateral connections and joint research projects with various Institutes". Cuba had taken 10 years to reach this stage. "But Iraq, Algeria and Nigeria have an economic basis", he added, "so they can reach the second stage more quickly".

This "tendency towards real reciprocity", said Mr Orosz, is typical of TESCO. Mexico, for example, has developed its own research in the growing of maize, a crop of considerable importance to Hungary also. A similar pattern, he explained, can be seen in Hungarian-Soviet co-operation. From 1948, when Comecon was established, until well into the 1960s, Hungary was largely the recipient. "We received some technology from the Soviet Union, and had

our experts trained there. We did have some co-operation, but it was on a 20-80 basis." By the mid-1970s, however, some 50 to 60 Hungarian research institutes were engaged in joint ventures with their Soviet opposite numbers.

Basic research and exchanges involving what Mr Orosz described as "concrete scientific themes" come not under the auspices of organizations such as TESCO, but that of the relevant Academies of Sciences. Nuclear research, for example, belongs here, and so does the Comecon space programme *Interkosmos*. The latter, it appears, (*Nature*, 280, 94-95) is financed overall by the Soviet Union, with the other member countries paying only for their own experiments. This would explain why Vietnam, which joined Comecon last year, could this spring send two cosmonaut candidates to Baikonur for training, although the Vietnamese economy is in such an urgent state that 75% of the proceeds of this year's *Subbotnik*, the Saturday in April when Soviet citizens contribute a day's work for the good of the economy, are to be devoted to Vietnam.

Many co-operation projects bring the Soviet Union and other developed Comecon partners gains in raw materials. The Comecon survey of Mongolia, for example, will allow Mongolia to increase its exports of minerals — presumably within the bloc. Some projects such as the Afghanistan and Trans-Iranian gas pipelines were constructed on a "buy-back" basis. Soviet oil and gas prospecting in India, Afghanistan and Pakistan, or coal prospecting in Nigeria might well be compensated in some of the product. On the other hand, recent agreements to build a hydroelectric station in Costa Rica or develop the cotton and wheat production of Angola will bring a less immediate return to the Soviet Union — except in good-will.

For it is impossible to assess the impact of Soviet/Comecon assistance to the Third World without considering political implications. Soviet histories of the United Nations intended for home readership present the Soviet delegates as engaged in a "struggle" for "progressive principles of technical assistance". The more moderate language of a Soviet report to the UN notes that "Economic independence of the developing countries is unthinkable without their scientific and technological liberation from imperialism...".

Unfortunately, goodwill projects can be affected by subsequent political changes. For many years, the Aswan High Dam, with its associated hydrotechnical projects and irrigation schemes was regularly quoted as a prime example of such an undertaking. Last May, however, on the fifteenth anniversary of the "changing of the course of the Nile", Moscow Radio's Arabic service could only speak sadly of "some persons" who are now striving to make the Egyptians forget the Soviet contribution to this project. □



Tent life: a display at the Mongolian exhibition now showing in London

Agroforestry: new hope for subsistence farmers

David Spurgeon reports from the first Conference on International Cooperation in Agroforestry, held in Nairobi last month

THE ancient practice of shifting cultivation has for many years been severely criticised by agriculturalists and foresters because of its destructive effects on both land and forests. Shifting cultivators fell and burn forest land, then plant food crops and raise animals and later move on when soil fertility drops. After a period of bush fallow, they return to the same site and repeat the process.

Historically, soil fertility was maintained first through the nutrients returned via the ash after burning, and later through natural processes during the long fallow periods. However, as population and economic pressures have increased, fallow periods have become progressively shorter and the land ever more exhausted of nutrients.

Within recent months, however, foresters and agriculturalists have been looking at these traditional practices with new eyes. Scattered but convincing evidence exists to show that the integration of trees, food crops and animal husbandry on the same piece of land can increase total productivity while preserving the ecosystem. Such a system could not only improve the way of life of the 250 million people in developing countries who depend on shifting cultivation for survival, but could help save the world's tropical forests from destruction. The name given to this approach is agroforestry.

The first Conference on International Cooperation in Agroforestry, held in Nairobi, Kenya, at the end of last month, was an indication of the widespread interest the concept has generated: delegates from 31 countries and eight international agencies attended. They strongly endorsed agroforestry practices, particularly in marginal or degraded areas where agriculture alone is not productive. They urged the extension of research into agroforestry systems throughout the developing world. And they recommend the adoption of education, training and extension programmes by appropriate institutions.

Much conference discussion was devoted to definitions of agroforestry. ICRAF's definition is a "sustainable land management system which increases the overall yield of the land, combines the production of crops (including tree crops) and forest plants and/or animals simultaneously or sequentially on the same unit of land, and (which) applies management practices that are compatible with the cultural practices of the local population." It was considered by some as not comprehensive or precise enough. No agreed-upon definition emerged: the

conference left it to ICRAF to hammer out a final version.

The lack of an adequate definition did not inhibit enthusiasm for the practice itself, however. Numerous examples were given of its benefits. One, from Soekiman Atmosoedaryo, president of Indonesia's state forest corporation, Perum Perhutani, was a programme that aims at developing forests not only as providers of wood and cellulose and protectors of the environment, but as sources of food, animal feed, honey, medicinal herbs, pine resin and natural silk. The programme, which involves growing rice between young forest plants, has raised paddy production from 0.7 tonnes per hectare to 1.8 tonnes within two years.

The director-general of the United Nations Food and Agriculture Organisation had told the 1978 World Forestry Congress in Jakarta that the forest areas of developing countries are being destroyed at the rate of 16 million hectares a year. At the Nairobi conference, a paper by Professors Jon Morris and Keith Openshaw of the University of Dar-es-Salaam translated this into starker terms: "Every three years an area of forest is being lost that could supply in perpetuity the wood raw material requirements of 480 million people — enough to meet all the requirements of the African continent!"

Peasants and subsistence farmers in developing countries are on the horns of a dilemma, according to Morris and Openshaw. On the one hand population pressure, rising expectations and faulty agricultural practices are causing them to destroy the woodlands. On the other hand, they need the products of the forest for cooking, heating and shelter, so they must preserve the trees. "A combination of agricultural practices are causing them to most logical way to do this," they said, "— having one's cake and eating it. And yet it is not universally practised."

Morris and Openshaw examined the economics of growing trees for fuel and poles together with agricultural crops in Kenya, and concluded that it "appears to be a sound economic proposition". The cost of planting a half hectare of trees would be only 740 Kenya shillings (about £49) over a 10-year rotation, with an average yearly cost of 70 Kenya shillings (\$4.7) while the financial yield would be approximately 12%, "well in excess of the minimum 8% that the Tanzania government expects from projects it invests in."

They concluded. "The farmer by growing his own wood products guarantees his energy, building materials and fencing



Integrating trees and animals: part of Indonesia's forest corporation

requirements as well as improving his microclimate, soil fertility, fodder and food supply. He may even produce a surplus to earn cash income."

One of the surprising revelations of the conference was the extent to which agroforestry practices are already being carried on throughout the world, and the amount of research already being conducted. Countries in which agroforestry is extensively used include India, Bangladesh, Sri Lanka, Thailand, and the Philippines in Asia; Trinidad, in the West Indies; Mexico and Costa Rica in Central America; Argentina, Brazil, Chile, Colombia, Ecuador and Peru in Latin America; and many countries in Africa, including some in the Sahel.

Agroforestry research programmes were described in a number of these regions. Dr Gerardo Budowski of Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE), Costa Rica, said "agroforestry has made an imposing start in the region (Central and South America), and will undoubtedly move ahead, particularly in wet areas where trees seem to be particularly indispensable ingredients. However, at this stage, it appears that scientists and administrators are more moved by wishful thinking than hard evidence. The task of designing appropriate technologies for specific ecological, social and economic conditions still remains to be done."

Dr Budowski's concern about the need for hard data was a central one at the conference. Yet some projects are already trying to obtain it. Besides those undertaken by CATIE, one was described by Abdul Malik Choudhury, deputy chief conservator of forests for Bangladesh. A pilot scheme is underway there to settle 300 families through agroforestry practices. Each family is given five acres of land on which to grow bamboo and practise

horticulture. They are paid for land clearance and given loans for inputs. A target of 9,000 acres is to be settled in this way in ten years, with an assessment after five years and an ultimate goal of 2.5 million acres if results of the pilot scheme are positive.

Although enthusiasm ran high for agroforestry, many voiced concern that too much might be expected of it, and were

cautious about the prospect of its acceptance as a new discipline. One question raised was whether promotion of agroforestry practices would condemn subsistence farmers to a life of poverty. Dr King, whose agency went into operation only a year ago, answered that "Without inputs (eg fertilisers) they are condemned to a life of misery and mere subsistence. Don't think that if we could get them

inputs we would be sitting here. You can grow anything anywhere if you have the money. It is precisely because farmers will remain at this level that some of us believe we could help them in a way that might not be so costly.

"Agroforestry is not the answer to all social problems. But I really think that, given the problems in some of these countries, it ought to be tried." □

Peer seeks to improve UK animal research law

LORD Halsbury's Animal Protection Bill successfully passed its first reading in the House of Lords on 16 July. Sometime after the start of the new parliamentary session on 23 October, it will receive its second reading. If it survives that hurdle, it will then be the subject for discussion of a Lords Select Committee before it is given its third reading and eventually handed over to the House of Commons for adoption as law — a procedure which could take up to two years to complete. Earlier this week, the Research Defence Society — which exists to promote the need for research with animals — held a press conference to mark the Bill's first successful reading.

Lord Halsbury, who has been President of the Research Defence Society for twenty years and has been interested in the use of animals in research for much longer, decided to formulate and sponsor the new Bill about one year ago. A series of events had persuaded him that it was time the government "took over its responsibility" to research and opposed the allegations of cruelty to animals in many laboratories being expressed in some of the tabloid newspapers. His Bill is intended to replace the increasingly criticised Cruelty to Animals Act of 1876 as the main piece of UK legislation for ensuring the protection of laboratory animals.

Two events last year convinced Lord Halsbury that the 1876 Act — which, he says is "mostly criticised by those who don't understand it" — needed revising. One was an address to the Annual General Meeting of the RDS in which the speaker expressed his growing distaste at experimenting with animals. ("I realised that I felt that way myself", says Lord Halsbury). The other was last year's Labour Party conference which was attended by a number of slogan bearing anti-vivisectionists and resulted in the Labour government promising to set up a Royal Commission on Animal Protection to look at all aspects of animal rights (a promise which went by default when Labour lost the last election). Setting up a Royal Commission to tackle the problem would have been like "using a sledge hammer to swat a gnat", according to Lord Halsbury. The problems of animals on farms, in slaughter houses, in transit, in laboratories and as pests are all very

different. In attempting to cover all five areas, the Royal Commission would be trying to be a "council of perfection".

In spite of his belief that the 1876 Act does work reasonably well, Lord Halsbury has come to the view that there is a need for more restrictive legislation. "Scientists must realise that they cannot live on the 1876 Act forever", he says.

His main criticism of the old Act is that it is too narrow; so his Bill is designed to extend the scope of legislation beyond vertebrates to include other species as and when protection is considered necessary "in the light of advancing scientific knowledge of the nervous systems of such species". Current legislation caters well for "cuddlesome pets". "But the octopus doesn't get protection. This is a highly intelligent creature — at least as intelligent as some of the minor vertebrates." Arthropods, such as crabs, also do not receive protection under the 1876 Act. "Do they have pain nerve endings under their shells?" The incorporation of new species on to the protected list would, under Lord Halsbury's Bill, be up to the relevant Secretary of State.

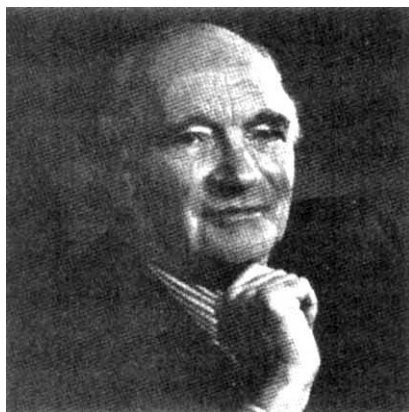
The new Bill would also extend the definition of a laboratory and an experiment. "All laboratory usage is included, even a green field". The word experiment has been deliberately excluded from the text of the Bill and replaced by "procedure" which should increase the number of animals entitled to protection. Lord Halsbury expects that the effect of this broadening of scope will be to increase the

number of institutions applying for licences to work with animals.

The overall philosophy behind the Bill is not very different from that behind the 1876 Act. After all "nothing that's said now is any different from what was said then", according to Lord Halsbury who has been studying old Hansards to find out the arguments in vogue before the introduction of the first Act in the 1870s. There are differences in some of the detail, however. For example, the Bill gives power to the Secretary of State to "make regulations to control the procurement, breeding, transport, accommodation, management, veterinary supervision and care of animals for laboratory use". It also gives stronger powers to inspectors, including the right to enter premises licensed under the Bill and to order the killing of any animal thought to be suffering unduly. The Secretary of State would also be required to publish a 'guide to good laboratory practice' which would include advice on the use of alternatives to experiments with animals. The Advisory Committee to the Home Office would be extended to include advising the Secretary of State on novel or controversial procedures and on the incorporation of new species.

For the success of his Bill, Lord Halsbury believes that he must have the support of the scientific community. The Select Committee stage at the House of Lords should provide scientists and others with the opportunity to study the Bill and express their opinions. But the Bill should only be a "little more restrictive" for scientists ("there are very few people who do experiments on octopus"). The anti-vivisectionists, however, are bound to be critical. "The militants will misrepresent the Bill. They will say that the word 'experiment' was cut out to disguise what scientists are doing, whereas procedure has been used to get animals protected".

The UK has amongst the toughest legislation of any country on the protection of laboratory animals and Lord Halsbury believes that it should take a lead in the Council of Europe's deliberations to formulate European guidelines. "The government says let's wait and see what the Europeans do. But the UK should take the lead. I make up my own mind first and other people's later". **Judy Redfearn**



Lord Halsbury: seeks support of scientists

news in brief

Tropical legumes can aid Third World economies: A report issued by a panel of the US National Research Council describes almost 200 species of underexploited tropical legumes that could prove valuable to "the economies of developing countries." The legumes, which include root crops, pulses, fruits, forage crops, fast-growing trees, luxury timber and fibre and gum producing legumes, can reduce dependence on costly chemical fertiliser, widen the range of agricultural products and increase the production of food proteins. Of the thousands of known leguminous species only 20 are used extensively. The 200 plants selected by the panel could produce edible oils, nutritious forage, paperpulp and firewood as well as resins, tannin, spices and food. The panel notes: "Of all plants used by man, only the grasses are more important than the legumes. However while enormous resources have been expended in recent decades on grasses like rice, wheat, corn, sorghum and barley, among the legumes only soybeans and groundnuts (peanuts) have received much attention." The report is critical of the 'green revolution' for not having decreased protein malnutrition in the Third World and calls for an equal effort to be spent on the production of pulses which "could quickly eliminate protein malnutrition for the immediate future."

J.P.M. Brenan, Director of the Royal Botanic Gardens at Kew near London and a member of the NRC panel cautions that there are difficulties to be solved before legume production becomes a reality. These include studies to determine whether plant breeding techniques can work to select good strains, whether the plants can be adapted to arid and semi-arid tropical environments and whether the plants could become pests when introduced to new areas. "It's a very large programme and we are too small and too poorly funded to carry it out quickly" he said.

UK research and development funding falls below 1964 levels: Research and development expenditure trebled in the UK between 1964 and 1975 but fell in real terms, according to an article in *Economic Trends*, published by the Government Statistical Service. Using 1975 price as a base, the article says £2,200 million was spent on R&D by government and industry in 1964 but only £2,151 million in 1975. The number of people employed in R&D has also fallen. In 1967, industry had 219,000 researchers compared with only 180,600 in 1975.

R&D expenditure peaked at the end of the 1960s and steadily fell from 1970 to 1975. Industry's expenditure fell particularly sharply in the early 1970s, whereas central government R&D expenditure rose between 1970 and 1975 but then fell from 1976-1977. In volume terms, however, R&D carried out by the government changed little, because between 1970-1975 the government financed more R&D actually carried out by other sectors. Defence research suffered the biggest cuts in central government spending falling from 0.86% of the gross domestic product in 1964 to 0.16% in 1977.

Wider brief for cholera lab: The Cholera Research laboratory in Bangladesh has been re-named the International Centre for Diarrhoeal Diseases Research, putting an end to last year's rows over the laboratory's future. Research and training on the diarrhoeal diseases and their related nutrition and epidemiology will be carried out at the centre, and finance and organisation will be multi-national. David Bradley of the London School of Hygiene and Tropical Medicine said he was pleased the work done at the Cholera Research Laboratory would continue. "It is a lab which has done superb work in the past and much of our knowledge of cholera has come from there. Dacca is a good place to have the new centre. There are over 100,000 people to work on there in an area where cholera is highly endemic." The laboratory had been reconstituted on a firmer international basis in order to expand its remit and funding.

China begins construction of its 50 GeV synchrotron: Surveying has begun near the Ming Tombs, Changting County, Beijing (Peking), for construction of China's first 50 GeV proton synchrotron. A preliminary design for the machine has been completed after several years of effort by 500 scientists and engineers in China. The final decision to build the facility was announced by vice-Premier Fang Yi in March 1978.

Since then about 100 factories and scientific institutions have been producing parts for the synchrotron. The Ministry of Metallurgy and the first and fourth machine-building industries, the High Energy Physics Institute of the Chinese Academy of Sciences and the Beijing Electrical Heavy Machinery Plant have already produced some of the required equipment.

China continues to push towards increasing its scientific and technical relationship with the West. At the Canton Trade Fair in April, China offered its nuclear instrumentation for sale abroad for the first time. A total of 14 equipment categories including instruments for handling radioactive isotopes and for measuring radioactivity, and remote handling devices, piping and valves for nuclear industry are being sold by the China Nuclear Equipment Corporation.



UKAEA launches informational campaign: The UK Atomic Energy Authority is opening a nuclear power exhibition in London beginning on 20 September for two weeks. Designed to take the "myth and mystery out of nuclear power" the exhibition will show that nuclear power is a tried and proven technology now providing 14% of the UK's electricity at a price that "industry can afford." A UKAEA official said the exhibition will present the facts about nuclear power with no need for a "recourse to opinion." The official told

Nature that there will be no information about Three Mile Island in the exhibit as "UK reactors are not of that type." The Secretary of State for Energy will open the exhibition and scientists and engineers will be on hand to answer questions. A large number of schools is expected to organise parties to the exhibition.

NRC invites public submissions on Three Mile Island: The US Nuclear Regulatory Commission is inviting the public to help it decide a complicated legal question of liability in the Three Mile Island accident of last April. According to the provisions of the Price-Anderson Act claimants for damages caused by an "extraordinary nuclear occurrence" do not have to prove negligence on the part of the company if there was (a) a substantial release of radiation off-site and (b) there were substantial off-site damages. If the commission were to make these findings a claimant would only need to prove injury or damage, the value of the damage and that the damages were caused by the accident. If the commission does not make these findings, the claimant has to prove not only damages but also that the company was negligent. NRC staff have given preliminary advice that the radiation released appears to be several hundred times less than the criterion set in the Act.

Eruption over Atlantis: Soviet oceanographer Andrei Aksenov announced recently that Western news agency reports last March of Soviet claims to have discovered Atlantis were a "misunderstanding". The underwater photographs which gave rise to the reports (of walls and buildings) now prove to show Mount Ampere, a submerged volcano some 380 km south west of Portugal.

correspondence

Dangers of low doses of radiation

SIR, — David Dickson's account (19 July, page 180) of the controversies surrounding the BEIR III report is evidently a fair and unusually competent job of science reporting. However, it should be made clear that the disagreement on the validity of the "linear hypothesis" when applied to carcinogenesis by low LET radiation does not, strictly speaking, concern the "filling" of a "gap". This terminology might be interpreted to indicate an interpolation while there is in fact the need for (much more uncertain) extrapolation. It is the magnitude of the risk at about 0.1 Gy (10 rad) that is in dispute. As stated by Dickson, there is agreement on even greater uncertainties at lower doses. I, for one, could not object to various surmises (including linear extrapolations) on what might happen below that level in view of the virtual absence of acceptable data on the carcinogenetic effects, if any, of such small doses.

By contrast, information on high LET radiations, and especially the data for Hiroshima, where neutrons plainly posed the principal hazard, indicates approximate linearity down to doses less than 0.1 Gy.

It should also be borne in mind that any fit of the data by linear or quadratic functions is probably at best a first approximation. Animal experiments show complicated — and sometimes inverse — correlations between radiation dose and carcinogenesis which vary not only between species and strains, but also between types of cancer. It would seem unrealistic to assume that the same does not apply to man.

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Nautilus records Earth's second moon

SIR, — I quite understand the need to close the debate on the fascinating Kahn-Pompea-Runcorn dialogue concerning the possibility that *Nautilus* has quietly recorded the lunar recession rate through the ages (31 May, page 452). However, it should be noted that (as in many debates) the participants, including *Nautilus*, may be arguing from different premises. That is to say, while Pompea, Kahn, and Runcorn are clearly discussing our present moon, let us say Luna, *Nautilus* may in fact have recorded the recession of a sister moon, let us say Selena, from Ordovician until rather recent geological time.

If Selena had been in orbit at 12 Earth radii in Ordovician time, as Pompea and Kahn propose for Luna, she could have dominated the *Nautilus* recording apparatus even with a mass much less than her sister Luna, who could well have been orbiting far out near her present position of 60 Earth radii. Perhaps a later Precambrian continental emergence had greatly increased tidal friction, so that light-weight Selena, receding from the Earth at the required ten-times greater rate than that of present-day Luna, finally approached her sister too closely, and was either devoured by

collision or ejected to the firmament. In any event, a small Selena could have orbited the Earth more closely, and have retreated much more rapidly than her larger sister, without dumping the excessive heat noted by Runcorn into the Earth-Luna system.

If Selena vanished before mid-Tertiary, then Runcorn's objection that there is no record of the maximum 33-day month which Luna should have produced at 45 Earth radii is of no concern; Luna would have been far beyond that orbit when she took over regulation of the Nautilian cycle, leaving every vestige of Selena forgotten except for a few shells and perhaps a very young lunar mascon.

It is worth noting that H. Alfven and G. Arrhenius (*Evolution of the Solar System*, NASA, 1976) have proposed, by analogy with the Martian satellites, that Earth once had a second moon at a predicted orbital distance of 13.5 Earth radii, very close to the inferred Nautilian Ordovician value of 12. Perhaps Selena finally perished simply because Earth had oceans of water; the persistence of two Martian satellites may thus mean that Mars has never had an ocean at all.

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Nuclear power: an evil necessity for some countries

SIR, — In a letter under the title "Nuclear power does not lead to nuclear weapons" (5 July, page 10), Mr Greenhalgh, speaking for the nuclear lobby group "A Power for Good", argues that there is no direct link between peaceful nuclear power and nuclear weapons. It is tragic that in this day and age such absurd views should be advanced, which fly in the face of convictions held by the majority of nations. The Non-Proliferation Treaty and the safeguard systems of the International Atomic Energy Agency are the best evidence of the concern which people all over the world have for years felt about the link between nuclear power and nuclear weapons. More recently, and in recognition of the weaknesses and shortcomings of the NPT and the safeguards systems, a number of new initiatives were taken. The London Club was set up to provide additional guarantees. The International Nuclear Fuel Cycle Evaluation, with 56 nations participating, is still studying ways and means of reducing the danger of proliferation arising from the use of nuclear power. In the United States a special act was brought into being for the same purpose. Why would this enormous effort be needed if there were no danger of nuclear power leading to nuclear weapons proliferation?

Mr Greenhalgh bases his argument on past history, that countries developed nuclear weapons before starting nuclear power programmes. But the fact that something has not happened in the past cannot be used as an argument that it will not happen in the future, particularly if the opportunities for it were greatly increased. One of the chief characteristics of beings endowed with thinking power is that they can look ahead,

anticipate possible trouble and take steps to prevent it.

A nation could, of course, obtain nuclear weapons by means other than power reactors, but the existence of one danger does not justify the introduction of another danger. Moreover, the setting up of a nuclear power programme, while not being a pre-requisite, is the most likely route to the acquisition of nuclear weapons, because the technology and the materials become legitimately available to a nation possessing nuclear facilities, including a reprocessing or an enrichment plant.

Mr Greenhalgh points to the existence of 100,000 MWe of nuclear power in the world as evidence of no direct link with nuclear weapons, but he forgot to add that this contributes less than 1% to the world's annual energy consumption. The situation would be radically changed if nuclear power increased by two orders of magnitude, as it would have to do (to meet the energy needs of the developing countries) in order to become the chief source of energy as envisaged by Mr Greenhalgh and his ilk.

Fortunately this is not likely to happen. The danger of proliferation, together with other well known negative aspects of nuclear power, as recently illustrated by the Three Mile Island accident, have now convinced public opinion that, at most, nuclear energy should be considered an evil necessity for some countries. Except for those with a vested interest, very few now believe that nuclear energy is a power for good.

J. ROTBLAT

London, UK.

Democracy cannot be defended by security police

SIR, — "Just imagine yourself sitting there below the judges' table . . . squashed like a lemon". 'Just tell us, are you a communist?'. 'What do you think of the GDR?'. 'If as a christian you are an anti-fascist — should you not be anti-communist as well?'. 'As a christian, you say love your neighbour. But how about the fascists?'. "

These quotations come from the official report mentioned in *Nature* (31 May, page 363), which to your correspondent has been "cleverly invented by leftist totalitarians". They are in fact excerpts from the questions asked of Heinrich Haberlein, a pacifist who was found by the constitutional courts unfit to be a teacher, because "while not belonging to those groups hostile to the constitution, his employment could be rejected, if he, while not actively combating the free and democratic constitution, could be reasonably supposed to be simply indifferent towards the latter." (Page 37 of the DGV report). I suppose that this is an example of what your correspondent calls the 'right to select', and it is of little surprise that the German Society for Behaviour Therapy's report carries the results of this policy.

Your correspondent seems to miss the point that you don't defend democracy by creating a vast security police to hound out 'totalitarian radicals' because you intimidate and pollute its concept.

C.N.M. POUNDER

Edinburgh, UK

news and views

Stops and starts in microtubules

from Dennis Bray

THE conventional wisdom that microtubules run the entire length of nerve axons and dendrites probably comes from a paper by Weiss and Mayr (*Acta Neuropath.* supp V, 198; 1971). On page 200 these authors conclude that "each tubule is a single unitary entity, extending in uninterrupted and undivided continuity from its base in the cell body to its peripheral tip". Clear enough one would think, but alas, the data are far less so. The evidence amounts to no more than the approximate constancy of tubule number in cross sections of six rat motor axons taken before and after bifurcations — a result that could obviously have arisen in several ways. Tubular continuity was presumably chosen as the explanation because it was consistent with the prevailing view of axonal dynamics. Tubules were believed to be synthesised at the soma and pushed out into the process; they were the continuous rails on which the fast transport ran. The whole story made such a neat package that it would have been churlish to worry about such corollaries as that the tubules in many parts of the mature nervous system would then be metres long. Best not to think of the recurrent laryngeal nerve of the giraffe.

Questions of continuity and point of origin also exist, although in a less extreme form, in non-neuronal cells. In fibroblasts, microtubules wander within the cytoplasm and can be seen by immunofluorescence as fine threads in tangled skeins. The method is sensitive enough to detect single tubules (Osborn *et al.* *J. Cell Biol.* 77, R27; 1978) but cannot resolve those in bundles. In consequence it is impossible to say in most preparations where the tubules begin and end. In 1976, however, three laboratories described a way to cut this Gordian knot. The tubules are depolymerised with colchicine or other antimitotic agent, the drug is washed out, and the cells fixed as the tubules begin to grow back again and then stained with antitubulin. When this is done the reforming tubules are first found in discrete star-like bodies in the cytoplasm, usually close to the nucleus (all of this applies to interphase cells; mitosis is different). The tubules lengthen from these centres until eventually the original network is regained. There is usually one,

sometimes two, of these centres in a cell and it seemed most reasonable to suggest that they were the centrioles or cytocentres which have sometimes been seen by electron microscopy within arrays of microtubules in interphase cells. The implication was then that in such cells all tubules lead to the cytocentre which serves as a nucleation point for their growth. This was analogous to the role of the nuclear assembly point envisaged for the nerve cell.

The picture at this time was simple and satisfying. There were one or two structures in a fibroblast, epithelial cell or neurone which are responsible for the initiation and subsequent growth of all the microtubules in that cell. A simple picture, satisfying — and shortlived. A few months ago in *Cell* (16, 239; 1979) Spiegelman and colleagues showed that a minor variation in the staining procedure can produce not 1 or 2, but 5 to 10 centres in the average fibroblast, all close to the nucleus and each with 10–30 radiating microtubules. The main change in procedure was a previous extraction with non-ionic detergent and the new result was attributed to the loss of background staining — the new centres emerging from the background like stars at dusk, so to speak. Even worse, the same approach when used on human fibroblasts or epithelial cells showed one or two major centres together with many single points so small that they seemed little more than individual tubules growing in the cytoplasm. There are a number of imponderable factors, as always. Is it possible that the preparation of cytoskeletons might disrupt the organising centres and scatter the newly growing pieces through the gutted cell? Or, to go back a step, how do we know that the recovery from antimitotic agents will follow the same course as growth in a healthy cell? Within the limitations of the method, however, the picture obtained by immunofluorescence is of multiple nucleating centres, possibly of several distinct types.

Back at the nerve cell, a cultured neuroblastoma when treated by the above procedure shows a single organising centre at the base of its one or two neurites (Spiegelman *et al. op. cit.*). Dividing neuroblastomas look more like a fibroblast in that they have an average of 12 smaller sites distributed around the nucleus. Obviously as the cell 'differentiates', the multiple

centres coalesce to a single one and from a timed series of specimens Spiegelman *et al.* show that this occurs, on average, before the neurite grows out. The intriguing possibility is thereby raised that this coalescence in some way instigates neurite outgrowth. At any event, the tubules in this study are still firmly anchored to organising centres, small or large, in the soma.

The hint that even this tenet might be on shaky ground existed in an ultrastructural analysis of crayfish nerve (*J. Neurocytol.* 3, 73; 1974). Nadelhaft found that sections of the same axon at different points along its length could show a significant variation in microtubule number. His polite, but firm conclusion was that the hypothesis of microtubular continuity could not be universally valid. A conclusion that received a thumping validation in the July issue of the *Journal of Cell Biology* (82, 278; 1979). Here Chalfie and Thomson present a serial analysis of certain axons in the world's most photographed worm. These axons are notable for their distinct tubules which are larger than tubules elsewhere in the animal and very highly ordered. Serial sections show major fluctuations in tubule number along the length of these axons and frequent terminations. The tubules are in short lengths, between 6 and 27 μm , distributed in staggered arrays along the axon. Even more interesting is the observation that the two ends of each tubule are not the same. The distal end, furthest from the cell body, terminates in a cloud of light staining that is always right up against the plasma membrane. The proximal end can be anywhere in the cytoplasm but is characterised by central density in the tubule. Assuming that these features are not freaks of these specialised axons — and breaks can be found in other tubules in the animal — the Weiss and Mayr picture of continuity is roundly wrong. And the question of how microtubules grow, and from where, in neurones as in other cells, is back to the starting point. Perhaps the rabid reductionists are right and we will have to wait until the analysis of tubulin polymerisation *in vitro*, under controlled conditions and with defined components, provides the answer. There have been many such studies and they have revealed a rich and multifarious set of phenomena. It is true that they have yet to point to a single mechanism, but that is another story. □

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Conformational flexibility in protein molecules

from Robert Huber

In describing protein molecules the term flexibility is used with quite different meanings. A precise definition for a particular protein molecule would require the determination of the number, energy and geometry of the various stable conformational states the molecule might have, the kinetic parameters of interconversion and the thermal motion of all the atoms in each conformer: a considerable task, requiring a great variety of experimental techniques, if feasible at all. Several rather different experimental approaches have provided evidence that protein molecules are indeed flexible: deuterium-hydrogen exchange under non-denaturing conditions¹, fluorescence quenching of internal tryptophan residues by oxygen diffusing into the protein molecule², ESR, NMR, and fluorescence depolarisation. These last spectroscopic techniques allow one to determine the mobility as a function of temperature of certain residues whose spectroscopic signal can be identified. The power of these methods is clearly demonstrated by recent experiments, using NMR techniques, which show that the aromatic rings of tyrosyl and phenylalanyl residues in pancreatic trypsin inhibitor (PTI) flip³⁻⁵. Model calculations show that aromatic ring flipping is a consequence of substantial breathing motions of the protein, which include movement of the polypeptide backbone^{6, 7}. The mobility of tryptophanyl residues has been probed, using very short and intense light pulses to measure fluorescence depolarisation⁸. Motions in the picosecond time range are found, but the degree of flexibility is different for different protein species, some being rather rigid, some soft. Recent molecular dynamics calculations on PTI⁹ show structural fluctuations in the picosecond range of a size in rough agreement with the observed crystallographic temperature factor¹⁰ and promise to provide the theoretical background to motions in proteins. However, the computational problems are enormous and, so far, allow the analysis of a small protein molecule such as PTI for a few picoseconds only. This makes extrapolation to equilibrium properties and comparison with the time-averaged crystal structures problematic, as does the neglect of solvent effects in calculations performed to date. More refined dynamics calculations are currently being pursued.

Protein crystallography as a means to gain information about flexibility is unique

in providing us with an overall view of the molecule in contrast to the spectroscopic techniques which focus on a particular label. However, there are a number of theoretical and experimental problems. As the physical diffraction process is instantaneous compared with lattice vibrations, the 'temperature' factor determined crystallographically does not differentiate between thermal motion and static disorder. Static disorder may have components from conformational micro-heterogeneity of the protein molecules (substates or microscopic states) and from lattice defects. The interesting parameters, thermal motion and substates are difficult to determine accurately and still more difficult to separate from one another. The experimental problem has been partly overcome since the development of techniques for refinement of protein crystal structures, some five years ago.

Protein structures were successfully refined¹⁰⁻¹² using principles borrowed from small molecule crystallography, extensively modified and adapted to the much larger problem¹³. From these studies it was obvious that the 'temperature' factors of individual atoms were important variables for the correct description of the protein structure. However, the values were regarded with some suspicion, as they changed from atom to atom in a rather erratic way due to the limited resolution of the diffraction data. But, when the 'temperature' factors were averaged for rigid groups and smoothed along the main and side chains, physically reasonable features were obvious: 'temperature' factors increased in external polypeptide loops and external side chains were more mobile than internal ones.

Several examples of protein crystal structures are now known where disorder (either dynamic or static) is a dominating aspect: antibody molecules^{14, 15}, trypsinogen¹⁶, citrate synthase¹⁷, tobacco mosaic virus protein^{18, 19} and tomato bushy stunt virus²⁰. In these molecules substantial parts show no significant electron density indicating disorder in the crystalline state. In some of the molecular species rigid states are known with different functions, providing evidence for the functional significance of large scale disorder²¹. Except in the case of TMV protein, where NMR experiments have demonstrated increased thermal mobility of the disordered segments²², it is unclear which type of disorder (conformational heterogeneity or thermal motion) prevails in these examples. Lattice defects involving the molecule as a whole are excluded, of course, as most parts of the molecules are well ordered.

Two reports in this issue of *Nature* are centred on the question of conformational disorder and its static and dynamic components determined by crystallographic methods in two different systems: lysozyme (Artymiuk *et al.* page 563) and myoglobin (Frauenfelder *et al.* page 558). Two different lysozyme species crystallising in different crystal forms have been carefully refined and atomic 'temperature' factors determined. When these were averaged for the main chain atoms of each residue a close correspondence between the two crystal structures was obvious, suggesting that the variation in 'temperature' factor along the chain is a molecular property and not seriously influenced by crystal packing. A detailed mathematical analysis indicates a rigid body translation and libration of the molecule as a whole, overlaid with additional flexibility of some external loops. Among these are the loops determining the substrate binding cleft in lysozyme. A positive correlation between ordered secondary structure and 'rigidity', and between shielding of amino acid side chains and their 'mobility' is obvious. The slightly enhanced 'mobility' of the substrate binding area is intriguing, in particular, if it were to be found that these segments 'rigidify' on substrate binding; one wonders why nature utilises such small effects, having in mind the drastic segmental flexibility in the examples discussed before. Artymiuk *et al.* do not and cannot dissect the 'temperature' factors observed into their static and dynamic contributions. This problem is the main object of the paper by Frauenfelder *et al.* who analysed and carefully refined

1. Woodward & Hilton A. *Rev. Biophys. Bioeng.* **8**, 99 (1979).
2. Lakowicz & Weber *Biochemistry* **12**, 417 (1973).
3. Snyder *et al. Biochemistry* **14**, 3765 (1975).
4. Wagner *et al. Biophys. Struct. Mechanism* **2**, 139 (1976).
5. Snyder *et al. Biochemistry* **15**, 2275 (1976).
6. Gelin & Karplus *Proc. natn. Acad. Sci. U.S.A.* **72**, 2002 (1975).
7. Hetzel *et al. Biophys. Struct. Mechanism* **2**, 159 (1976).
8. Munro *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 56 (1979).
9. Karplus & McCommon *Nature* **277**, 578 (1979).
10. Deisenhofer & Steigemann *Acta Cryst.* **B31**, 238 (1975).
11. Watempaugh *et al. Acta Cryst.* **B29**, 943 (1973).
12. Huber *et al. J. molec. Biol.* **89**, 73 (1974).
13. Diamond *J. molec. Biol.* **82**, 371 (1974).
14. Colman *et al. J. molec. Biol.* **100**, 257 (1976).
15. Ely *et al. Biochemistry* **17**, 820 (1978).
16. Huber & Bode *Acc. Chem. Res.* **11**, 114 (1978).
17. Wiegand *et al. Eur. J. Biochem.* **93**, 41 (1978).
18. Bloomer *et al. Nature* **276**, 362 (1978).
19. Stubbs *et al. Nature* **267**, 216 (1977).
20. Harrison *et al. Nature* **276**, 368 (1978).
21. Huber *Trends in Biochem. Sci.* (in the press).
22. Jardetzky *et al. Nature* **273**, 564 (1978).
23. Parak *et al. Acta Cryst.* **A27**, 573 (1971).
24. Epp *et al. Biochemistry* **14**, 4943 (1975).
25. Steigemann & Weber *J. molec. Biol.* **127**, 309 (1979).
26. Richards *J. molec. Biol.* **82**, 1 (1974).

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myoglobin crystals at various temperatures from 300 to 220 K, with the ultimate goal of differentiating static and dynamic disorder by their low temperature limit. As protein crystals contain solvent which may not be allowed to freeze, the temperature range that is covered is small as is the effect on the 'temperature' parameters determined.

The principal result of Frauenfelder *et al.*, that 'temperature' factors are increased in the peripheral parts of myoglobin and correlate with the secondary structural elements, agrees with the observations on lysozyme and other proteins discussed. The authors also follow and compare the variation of the 'temperature' factors with temperature for internal and peripheral atoms: they make the novel and interesting observation that internal atoms 'freeze' more than peripheral ones. This leads the authors to the view that conformational heterogeneity (substates) is a dominant type of disorder, at least for the outer parts of myoglobin. As the temperature range covered is small and the effects measured marginally above the level of significance, it would be highly desirable to back up these results by going to substantially lower temperatures, perhaps using the shock-freezing technique developed by Parak *et al.*^{23, 24}. These authors had shown by Mössbauer absorption spectroscopy on myoglobin crystals that about half of the crystallographic 'temperature' factor is due to static disorder. Mössbauer absorption

measurements are insensitive to static disorder as long as the equilibration between different conformers is slow compared with the life time of the excited Mössbauer nucleus. The question of whether a protein molecule is to be regarded as a vibrating aperiodic crystalline solid or a 'semiliquid' 'glass', characterised by a larger number of substates of similar energy, is a topic of lively discussion at present. Of course, there is clear evidence for alternative side chain conformations in protein crystals (see for instance Epp *et al.*²⁴ who observed conformational heterogeneity of an internal cysteine residue; or His E7 in erythrocruorin (Steigemann & Weber²⁵)). However, the uneasy feeling of a protein crystallographer about the 'semiliquid', 'glassy' protein picture comes from the fact that proteins are tightly packed²⁶, multiply linked and cross-linked molecules which may have problems finding efficient alternative packing except, of course, for residues and loops interacting with solvent. It would, perhaps, be appropriate to defer these arguments until it is possible to define more precisely the extent to which substates exist in a particular type of disorder. The differentiation between a single vibrating conformer and an assemblage of a huge number of closely related substates separated by low energy barriers and in rapid equilibration becomes a matter of semantics, except close to 0 K, a rather unphysiological temperature. □

reach the satellite, by which time it will have spread out in space as a result of the velocity dispersion. Reasonable assumptions show that it will take about 7 h to sweep past the spacecraft. The experiment was working between 7 February, 1972 and 2 August, 1974 and during this time 19 group bursts were seen, individual groups taking between 40 min and 13 h to pass the spacecraft. Fechtig, Grün and Morfill tried to correlate the observation times of the groups with the occurrence of seismic events on the Moon recorded by apparatus left behind by the Apollo astronauts. There were ten times more seismic events than group events. This fact is not too disheartening, however, for fast ejecta would leave the impact point at an angle of less than 10° to the surface, so only the lunar rim regions can provide ejecta detectable at the spacecraft.

The source suggested for the swarm bursts is the Earth's auroral zones. About 30% of the meteoroids in space with masses above 100 g have densities less than 1 g cm⁻³, and these meteoroids are thought to be fragile, crumbly, loose conglomerate bodies made up of collections of individual particles in the micron and even submicron range. In interplanetary space meteoroids are positively charged with potentials in the range from +3 to +20 V. The main cause of this is the photoelectric effect. Solar quanta of sufficient energy (more than about 10 eV) may dislodge electrons from the surface layers of the meteoroid and sufficient numbers of these electrons escape the Coulombic back-attraction to leave the meteoroid positively charged. This charge is somewhat reduced by the capture of solar wind electrons and there are other much less dominant effects such as proton capture, secondary electron emission, sputtering and thermionic emission that can be neglected.

However when this meteoroid enters the ambient plasma of the Earth's magnetosphere at an altitude of about 60,000 km it quickly becomes negatively charged. The dominant charging process here is collection of electrons and protons from the Maxwellian plasma. Remember that the thermal electron and proton velocities are much greater than the meteoroid velocity. The majority of electrons and protons that hit the meteoroid stick to it. Also a meteoroid of radius greater than 10⁻⁵ cm can completely stop electrons of energies less than 10 keV and protons of energies less than 70 keV. S.P. Wyatt (*Planet. Space Sci.* 17, 155; 1969) estimates that an iron, nickel or silicate meteoroid in sunlight at the Earth's orbit would be losing about 2.5×10^{10} electrons per second per cm² due to the photoelectric effect, so this has to be taken into account too.

Fechtig, Grün and Morfill consider two very dissimilar regions in the magnetosphere — first, the plasmasheet, a region outside the dipolar field regime. Here the electron number densities are

Meteoroid fragmentation in the auroral zones

from David W. Hughes

SATELLITE measurements of the micrometeoroid dust particles in the Earth's vicinity have produced many interesting results in the last two decades. One of the more fascinating observations has come from experiment S 215 on the HEOS-2 spacecraft. Not only did this experiment measure the normal sporadic flux of these particles but it also found groups and swarms of micrometeoroids which had apparently been produced as lunar ejecta, and also as the debris from the electrostatic disruption of larger meteoroids.

H. Fechtig, E. Grün and G. Morfill of the Max-Planck-Institute for Nuclear Physics, Heidelberg, have produced a detailed analysis of these results in a recent edition of *Planetary and Space Science* (27, 511; 1979). We can dismiss the sporadic particles fairly quickly. They arrive randomly, (as expected) the number observed per unit time obeys Poisson statistics (as expected) and the flux is what one would expect from the zodiacal dust cloud, allowing for the enhancement produced by the gravitational field of the Earth.

A sizeable fraction of the observed particle events did not occur randomly, however, but came in correlated bursts, leaving the researchers with the problem of finding the sources of the particles responsible for these bursts. Two sources immediately sprang to mind — because there were two types of bursts. Swarm bursts lasted for less than 40 min and the time interval between consecutive particles was less than 10 min. This was taken to indicate that the source was comparatively close by. Group bursts had durations between 40 min and 13 h and the time interval between consecutive particles was less than 6.5 h. The source of the particles responsible for the group bursts was obviously further away.

A meteoroid impacting on the Moon will produce a crater. Ejecta from this crater will consist mainly of lunar crust material, some of it having a velocity in excess of the lunar escape velocity, while the velocity dispersion will also be of the order of the escape velocity. The HEOS-2 satellite is in an Earth orbit with a perigee of 10,000 km and an apogee of 244,000 km. The lunar ejecta has to travel about 384,000 km to

between 0.1 and 10 per cm³ and the electron and proton energies are about 1 and 10 keV respectively. The second region is the inner magnetosphere, in the field line regime connected to the auroral zones. Here, especially during geomagnetically active times, the fluxes can be as high as 2×10^{10} particles cm⁻² s⁻¹ and the electrons have energies of about 40 keV. Although meteoroids seem to traverse the first region without harm, dust grains larger than 0.002 cm radius can be charged to very high potentials in the second region.

The authors estimate exactly how high this potential is from the HEOS-2 results. The internal disrupting stress, due to electrostatic repulsive forces, which acts on a meteoroid is proportional to the square of the ratio of surface potential to radius. For example, a surface charge of -500 V will break up a 100 μ m diameter dust ball, a 1 μ m diameter meteoroid made of compact stone or a 0.05 μ m iron particle. The fragments of this electrostatic explosion have a dispersion velocity inversely proportional to the square of the radius. Now the swarms observed by HEOS-2 are made up of fragments of radii about 10^{-5} cm (mass about 10^{-14} g) and the maximum time taken for a swarm to pass the satellite was 90 min. An analysis of the fragment dispersion indicated that the mean surface potential required to break up the meteoroids was about -600 V and 90% of the meteoroids producing swarms broke up at potentials in the range between -300 and -960 V. It was obvious that in most cases the breakup only took place after the meteoroid had entered the inner dipolar field region of the magnetosphere, the region in which intense fluxes of energetic auroral electrons have been observed. A particle of radius r will charge up to -600 V in 0.13 r^{-1} s on entering the auroral zone.

The swarm data indicate that the primary particles are interplanetary in origin. From the area of the detector, the exposure time and the total number of swarms observed, the authors have estimated what percentage of the large interplanetary meteoroids incident on the Earth's atmosphere actually break up as they pass through the auroral zone. The swarm parent bodies have estimated masses in the range 10 g to 10^6 g. When the flux of swarm-producing meteoroids is compared with the flux of similar mass objects that produce lunar seismic events and fireballs visible to the Prairie Network of all-sky cameras, it is found that only between 1 and 10% of the incident bodies actually fragment into micron and submicron particles at auroral zone heights.

The parent bodies are from the same group that produce type III fireballs having low densities (<1 g cm⁻³), low geocentric velocities (<20 km s⁻¹) and orbits with semi-major axes around 1 AU. So in these few

incidences the Earth's atmosphere is not hit by a single large meteoroid but is peppered by millions of submicron particles which will not produce meteors in the atmosphere but will simply be retarded and then drift slowly to the ground. Fragmentation takes place at a height of about 10 Earth radii. The average meteoroid observed was moving at around 8 km s⁻¹ when it broke up and the fragments produced dispersed at about 1 km s⁻¹, thus peppering an area of about 10,000 km in diameter, of the same order of magnitude as the cross sectional area of Earth.

Fechtig, Grün and Morfill are continuing their research and expect to see similar effects in the vicinity of Jupiter which has a strong magnetosphere and a superfluidity of moons. They have already designed a suitable dust experiment for inclusion in the Jupiter Orbiter Mission scientific package. □

Superfluid vortex waves

from P. V. E. McClintock

RADIO frequency vortex waves have been detected on quantised vortex lines in superfluid ⁴He, according to a report by R. A. Ashton and W. I. Glaberson of Rutgers University in a recent issue of *Physical Review Letters* (42, 1062; 1979).

Vortex lines form in liquid ⁴He if attempts are made to rotate a container of the liquid when it is below its superfluid transition temperature of 2.2 K. In this respect, as in many others, the superfluid behaves in a manner strikingly different from classical liquids. Whereas an ordinary liquid can rotate with its container much in the manner of a solid body (apart from acquiring a parabolic upper surface) superfluid helium is unable to rotate. In fact, for low enough angular velocities of the container, the superfluid apparently remains at rest relative to the fixed stars and does not rotate at all, no matter how long the experimenter rotates the container. If the container is speeded up, however, a single vortex line eventually appears; but the liquid still does not rotate as a whole. The vortex line is a microscopic quantal analogue of the eddy which is often seen as water runs out of a bath plug-hole; it is a line, parallel to the axis of rotation of the container, around which the liquid circulates with a tangential velocity which is inversely proportional to the distance from the axis, or core, of the vortex.

Speeding up the rotation of the container still further causes additional vortex lines to appear, all identical, because each one is

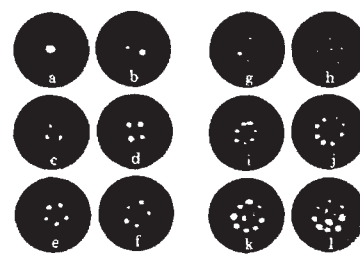


Fig. 1 Photographs of stable vortex arrays, looking along the axis of rotation. The angular velocities with which the 2 mm bucket was rotated varied from 0.3 rad s⁻¹ to 0.86 rad s⁻¹.

singly quantised. At 1 rev s⁻¹, there are about 10^4 cm⁻². Since each line exists in the flow field of all the others, the whole array of lines rotates at the same rate as the container, thus to some extent simulating solid body rotation of the liquid as a whole and producing what appears to be a conventional parabolic surface (although, on the microscopic scale, one expects dimples where the cores of the lines touch the surface). Support for this general picture comes from a number of sources, but the most direct evidence is from the beautiful experiments performed at Berkeley by Packard and his co-workers, who have managed to take photographs of the vortices. One of their latest results (Yarmchuk, Gordon & Packard *Phys. Rev. Lett.* 43, 214; 1979), reproduced in Fig. 1, provides a striking illustration of the structure of the rotating liquid.

The lines are potentially capable of supporting vortex waves: travelling helical deformations in which each element of line moves in a circle, though in the opposite sense to that of the vortex flow field. The phenomenon has been known for some time in relation to classical fluids, the first detailed study being due to Kelvin (*Phil. Mag.* 10, 155; 1880). Early attempts to observe vortex waves in helium were based on torsional pendula, and were restricted to frequencies below 1 Hz; they yielded somewhat ambiguous results. More recently, however, it has been suggested that vortex waves of much higher frequency might be both excited and detected with the aid of negative ions.

Much of our knowledge of the quantised vortices has, in fact, been discovered through ion experiments. The negative 'ion' in liquid helium — in reality an electron confined within a 20 Å radius bubble — is easily trapped on a vortex line. Although it cannot escape in the direction perpendicular to the line, it readily slides along it under the influence of an electric field and, by measuring the velocity of the ion as a function of field, it has been possible to deduce information about the properties of the vortex itself. (The photographs in Fig. 1 were taken by shooting ions right off the top of the vortex lines to strike a detector placed above the liquid surface, thus revealing the position of each line.) Halley and Cheung suggested

(Phys. Rev. 168, 209; 1968) that one way of exciting vortex waves might be to subject the line to a transverse oscillatory electric field while ions were travelling along it. This resonant excitation of vortex waves would have a corresponding effect on the motion of the ion. To achieve it, several conditions must be fulfilled. In particular, the ions should be moving along the line at the same speed as the propagation velocity of the vortex wave corresponding to the frequency of the applied electric field. Halley and Ostermeier were subsequently able to calculate in detail (*J. Low Temperature Phys.* 26, 877; 1977) how the velocity of the ion might be expected to vary with the d.c. electric field propelling them along the line. They predicted that there should be a kink in the velocity-field characteristic at a certain critical velocity, which depends on the frequency of the transverse oscillatory electric field.

Ashton and Glaberson have now performed this experiment, using a rotating ^3He cryostat in which the ^4He sample can be cooled to 0.3 K while being rotated at speeds of up to 10 rad s^{-1} . The transverse electric field was circularly polarised, so that resonant vortex wave production was expected to occur for only one polarisation for a given sense of cryostat rotation. Their experimental results are shown in Fig. 2. It is quite clear that the radiofrequency (RF) electric field has a considerable influence on the ionic velocity V , and that entirely different effects are obtained when the applied field is changed from a clockwise (CW) to a counterclockwise (CCW) sense relative to

the rotation of the cryostat. An anomalous kink and plateau occurs in the velocity at about 3.6 m s^{-1} , in tolerable agreement with a theoretical prediction of 3 m s^{-1} for the RF frequency being used (10 MHz). This result can be regarded as a vindication of the principal conclusions of the Halley and Ostermeier theory. But the theory does not give a satisfactory account of the detailed shape of the velocity-field curve; nor is it able to explain the reduced values of V found in the CW rotation.

A good deal more work, both theoretical and experimental, will be required to sort out the details. Even on the basis of the present results, however, it seems clear that high frequency vortex waves really do occur in superfluid ^4He . □

Erratum

In the article 'Hypercycles and the origins of life' in last week's *News and Views* (*Nature* 280, 445; 1979), two lines were inadvertently transposed from the foot of the second column on page 445 to the middle of the second paragraph in the third column. Starting from the last line of column 2 the correct reading is "Then before long only one quasi-species would remain — GOD only, or THE only, if short words replicate faster." and in the middle of paragraph 2, column 3, the correct reading is "I and I_2 are two RNA quasi-species. Both the + and - strands must be good replicators."

The cladistic debate continued

The critical view of the cladistic method of classification taken by L.B. Halstead in his report of a meeting on Vertebrate Palaeontology published in News and Views some months ago (276, 759; 1978) and the rejoinder by a group of supporters of 'cladism' (277, 175; 1979) provoked a large correspondence, a selection of which is published below.

There can be few disagreements on methodology in the recent history of biology which have been pursued with such passion by the protagonists, while leaving biologists not involved (in this case in systematics) so unmoved by, or even ignorant of, all the fuss. There is fruitful territory for psychologists here, but I would like to confine myself to the methodological rather than the theological aspects of the affair.

The fundamental tenet of cladism, is that in systematic biology the reconstruction of phylogeny is logically prior to the construction of a classification and that the latter is a redundant expression of the former. Unlike all other taxonomists, the cladists characterise phylogeny solely as the result of speciation or cladogenesis (the splitting of one species into two or more in evolution). They ignore the results of evolutionary change within evolving species (phyletic evolution or anagenesis). Furthermore in reconstructing phylogeny they allow division only by dichotomy: that is, speciation, according to them, is always the splitting of one species into two daughter species, sister species of one another. Primitive cladists held this as a dogma, which, with our current knowledge of evolutionary genetics, is patently absurd. Sophisticated advocates use it only as an operational principle. Nevertheless the rationale of the method depends on evolution by speciation which results in reproductive isolation between sexually reproducing organisms. Cladistic classification is therefore inapplicable to non-sexual organisms and will presumably never be used for microorganisms such as Protozoa, bacteria and viruses.

The recognition of sister species (or groups of higher rank) is by shared derived characters unique to the sister groups and thus presumed present in their (always hypothetical) unique common ancestral species. Shared primitive characters, those also present in collateral groups outside the pair, are ignored. Unfortunately if a large number of character states is considered, as it should be by any competent taxonomist, contradictions will arise due to convergently evolved characters mimicking true synapomorphies (shared derived ones). The method of resolving this difficulty destroys the whole rationale of the

method of recognition of sister groups. The inconsistencies are resolved by using the principle of parsimony. By parsimony cladists mean that the sister-grouping which yields the smallest ('most parsimonious') number of contradictory characters compared with the number of apparent shared derived characters, of those the cladist has studied, is the correct one. This preposterous assumption could only be true if, first, an organism (be it lungfish, salmon or cow) could be analysed into a finite number of discrete characters, and, second, if a competent cladistic taxonomist could be reasonably sure that the tiny number of shared characters he can study in that organism has a similar ratio of true derived to convergent characters as does the class of all its characters.

Admittedly, traditional and also phenetic ('numerical') taxonomists use parsimony in an informal way, but they also use the criterion of predictability, perfectly described in the last century by John Stuart Mill, as an extrinsic criterion to test their classifications. Despite the fact that many cladists pay lip-service to the hypothetico-deductive method usually associated with the name of Sir Karl Popper, their only test of a cladogram appears to be a comparison, based on parsimony, with other contradictory cladograms. It is perhaps for this reason that a number of incompatible schemes for the mutual relationships of the major groups of fish-like vertebrates have been proposed by cladists, without any successful criterion within the system to choose between them.

At the risk of earning the opprobrium of your cladistic correspondents, I should like to end by pointing out that the problem I have touched on was well known to that very pre-Darwinian taxonomist Aristotle. In *de Partibus Animalium* (Book 1, paragraphs 2-4) he notes that a dichotomous classification based on a single character state will inevitably lead to an artificial classification (as it did recently when one eminent palaeontologist used it in the fundamental division of the Class Mammalia), but that the use of several character states leads to contradictions which cannot be resolved.

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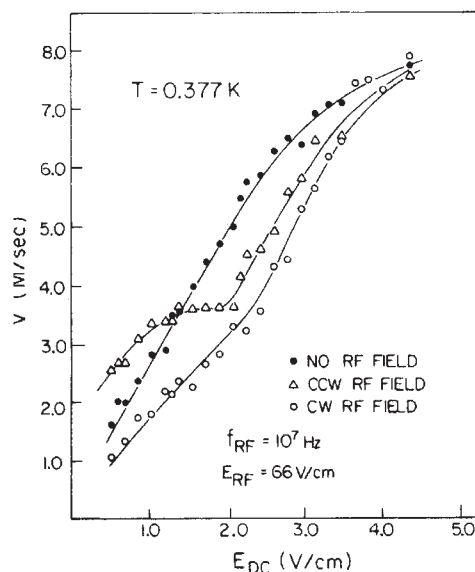


Fig. 2 Measurements of the ionic velocity V along a vortex line as a function of the longitudinal electric field E_{DC} : for zero applied radiofrequency (RF) field; and with the latter field polarised clockwise (CW) or counterclockwise (CCW) relative to the rotation of the experimental cell. The anomalous plateau and kink at about 3.6 m s^{-1} in the CCW curve is interpreted as evidence of a coupling of the ions to vortex waves.

Cladism defended

THE contribution of L.B. Halstead to your journal was so misinformed about the assumptions and benefits of phylogenetic analysis that we are compelled to comment. A thorough presentation of phylogenetics ('cladistics') is inappropriate, but we do wish to make some remarks.

Phylogenetic methods are used to determine the most parsimonious arrangement of the patterns of character distribution among taxa (groups of organisms). These patterns are assumed to be the results of evolutionary processes and to reflect natural, monophyletic groups. The result of the analysis is a cladogram, a branching diagram with each branch justified by a character thought to be a unique evolutionary innovation. It should be noted that these diagrams are in fact hypotheses about relative recency of common ancestry among the taxa considered. 'Relationship' is thus defined by a genealogical criterion rather than such vague concepts as proportion of shared genes, as evidenced by supposed morphological similarity, or estimation of overall morphological similarity.

Dr Halstead's remarks about the phylogenetic system being designed such that fossils cannot be included is simply uninformed: many phylogeneticists have dealt with this issue, including one of us (E.O.W.) who published a phylogenetic analysis of fossil and Recent gar fishes.

Dr Halstead seems to be most interested in those evolutionary changes that 'allow' organisms to enter new 'adaptive zones', concepts widely used by grade-oriented systematists. He states, "the fact that many parallel changes can be related to adaptations to similar conditions, is more significant than listing trivial trademarks from which cladograms can be constructed." But, with some reflection, that statement yields a simple fact: parallel changes in evolution are not discernable, indeed are incomprehensible, without a genealogical hypothesis of relationship. Put simply, how can we know that similarity shared between two organisms is a parallelism without a phylogenetic tree which demonstrates that the character was not, in fact, found in the common ancestral species of the two taxa? This concept is so basic that we find it difficult to understand the misconceptions concerning it. Further, the "trivial trademarks" which mark monophyletic groups are often the very characters which are associated with new "adaptive zones" (for example, feathers of birds and mammary glands of mammals).

Dr Halstead states that if one could trace the ancestry of man back in time to a rhipidistian fish, the whole sequence of changes would, by definition, be a single monophyletic species. He adds that "most zoologists and palaeontologists" would surely use arbitrary divisions to partition that lineage. This misses another basic biological fact. The lineage is broken up, from the Devonian to the Recent. If this were not so, we would observe only one tetrapod species, *Homo sapiens*, and not the diversity of land vertebrates that we observe today. (Wiley (*Syst. Zool.*, 11-21, 1978) has shown that conclusions such as Halstead's stem from an artificial concept of classification.)

Since "the issue was summed up for many by R. Parrington's exasperated exclamation that according to the cladists a lungfish is more closely related to a cow than to a salmon," it is appropriate to end with a comment on that statement. It is not the cladist who 'makes' a

lungfish more closely related to a cow than to a salmon any more than it is a cladist who 'makes' *Hyracotherium* more closely related to *Equus* than to Eocene condylarths. Evolutionary history as exemplified by genealogical descent with modification (a Darwinian concept) 'makes' it so. Lungfishes and cows are more closely related to each other than lungfishes are to salmon because lungfishes and cows share a common ancestor that is not shared with salmon. That this is somehow 'wrong' indicates a confusion on the part of critics about the meaning of genealogical relationship. If cladograms, with their clearly defined data and clearly stated hypotheses, "are difficult enough for experts in the field to comprehend fully," then we suggest that the shortcomings lie with the 'experts' and not with phylogenetic methods or their results.

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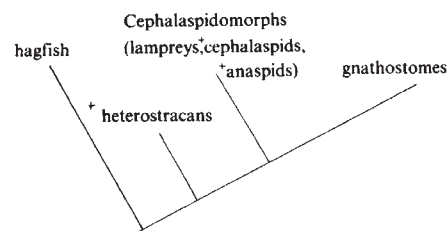
As a non-participant in the scientific meeting of which Dr Halstead's report has stirred such sharp reactions in your columns, but as a previous exponent (*Classification and Biology*, Heinemann, 1970) of the principles excoriated by Halstead under the name of 'cladism', I would like to address four questions to him. First, would he agree that there is not, and probably cannot be, an objective measure of 'degree of similarity' between organisms in respect of phenotypic characters of the type studied by museum systematists, and in particular that the 'unit character' of the numerical taxonomists has proved to be undefinable? Second, would he accept that the most fundamental type of similarity or difference between organisms is that of their genotypes, as represented primarily by their nuclear DNA? Third, would he accept that we now have objective methods of measuring DNA similarity, either directly (by DNA hybridisation and similar techniques) or indirectly (by comparative protein studies) and that the range and reliability of data from these techniques is likely to increase? Fourth, if by such methods it could be shown that a lungfish was 'nearer' to a cow than to a salmon, would Dr Halstead then be prepared to accept the 'cladist' case in this instance. And if the answer to this question were "yes", would this alter his general attitude to phylogenetic classification in the sense proposed by Hennig (and myself)?

R. A. CROWSON

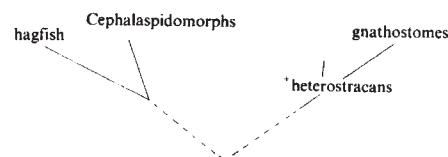
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HALSTEAD states that the phylogenetic ideas illustrated in the "...cladograms presented by P. Janvier (University of Paris) on agnathans and B. Gardiner (Queen Elizabeth College, London) on choanichthyans ... were demolished within hours of their being proposed". I wish to reply to this statement, which is designed to dismiss cladistics in general and Dr Gardiner and myself in particular.

I proposed the following phylogeny based on cladistic methodology.



An objection was raised by Dr Halstead who did not agree with the proposed affinities of the Heterostraci, a group of poorly known fossil agnathans. He pointed out that heterostracans probably had paired nostrils and nasal sacs (diplorhiny) and were therefore related to gnathostomes and not to other agnathans which are monorhinous. This information is supposed to demolish my phylogeny and to support his own 'evolutionary tree' given below (with permission):



We agree that the diplorhinal condition is a primitive craniate character-state. Therefore, Halstead must believe that primitiveness is a criterion by which we recognise relationship. As a cladist I cannot accept this view.

If heterostracans did have paired nostrils (and not all palaeontologists accept this interpretation) then I have to recognise that monorhiny arose twice; in the hagfish and in cephalaspidomorphs. To counter this objection I could lean on detailed embryological and anatomical information to argue that the hagfish monorhiny had arisen independently of that seen in the lamprey. But this approach is both fruitless and unnecessary in the light of other information which I presented. Furthermore, basing one's case on whether heterostracans were monorhinal or diplorhinal is a particularly unfortunate tactic. Heterostracans are represented by thin bony shields and the interpretation of the soft anatomy is governed by the recent model we choose. This is amply demonstrated by the differing views of heterostracan morphology proposed by Stensiö¹ and Halstead² for instance.

Unless Halstead can provide derived character-states to show that heterostracans are more closely related to gnathostomes than to other agnathans then I submit that the 'demolition' amounts to no more than a dislike of my idea because it differs from his.

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1. Stensiö, E.A. In *Current Problems of Lower Vertebrate Phylogeny*, Nobel Symposium 4, Almqvist & Wiksell (1968).
2. Halstead, L.B. *Biol. J. Linn. Soc. Lond.* 5: 339-349 (1973).

review article

Superheavy-element research

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The existence of an island of relatively stable elements beyond the present Periodic Table has been predicted by theoretical extrapolations of nuclear properties. During the past 12 years vigorous efforts have been made to discover these superheavy elements in nature and to produce them by nuclear reactions.

ALTHOUGH the existence of chemical elements much heavier than the known elements had already been considered more than two decades ago¹⁻³, these early studies did not receive much attention because for the heaviest known elements half lives in radioactive decay and production rates in nuclear reactions decrease steeply with increasing atomic number. Thus, there seemed no hope of proceeding to much heavier elements. In 1966, Myers and Swiatecki⁴ pointed out, however, that the stabilising effects of proton and neutron shell closures should be strong enough for certain elements beyond the explored Periodic Table to ensure a local stability against spontaneous fission comparable to or even greater than that of uranium. Furthermore, Meldner and Röper⁵ predicted that the next proton shell closure should occur at atomic number 114, a region not too far from the already synthesised heaviest elements.

The first detailed theoretical investigations of nuclear properties in this region⁶⁻⁸ revealed the topology of an island of relatively stable nuclei (Fig. 1) as a consequence of shell closures at atomic number 114 and neutron number 184. The elements forming this island are called³ superheavy elements. First estimates indicated half lives comparable to or even larger than the age of the Earth for nuclides in the centre of the island. Thus, superheavy elements could even exist in nature. The predicted long half lives encouraged many groups to search for such elements in terrestrial and extraterrestrial samples. The first negative results were published in 1969 by Thompson *et al.*^{9,10} who looked for element 110; the first positive results were reported for element 114 in the same year by Flerov and Pereygin¹¹ but later work showed that the background induced by cosmic rays had been underestimated in the latter measurements. First attempts to synthesise superheavy elements in the laboratory by fusion of ²⁴⁸Cm with ⁴⁰Ar projectiles had been reported a year earlier, again by Thompson *et al.*^{10,12}. In contrast to the search in nature, attempts to produce such elements by nuclear reactions can presently be carried out at only a few places, namely with the heavy-ion accelerators at Berkeley (USA), Dubna (USSR) and Darmstadt (FRG).

Here the most recent results will be discussed. Detailed treatments of earlier and recent¹⁶ work can be found elsewhere.

Nuclear and chemical properties

The decay mode ultimately terminating the Periodic Table will be prompt fission of the atomic nucleus. In terms of the well

established liquid-drop model of the nucleus, the stability of heavy nuclei is a delicate balance between two forces, the short-range attractive nuclear force between nucleons and the long-range repulsive electrostatic force between protons. In the heaviest known nuclei the attractive force dominates slightly, leading to a fission barrier which keeps the nucleus together. However, an excitation energy of only about 6 MeV, small compared with the total potential energy of 1,800 MeV of such nuclei, is sufficient to overcome the fission barrier, as evidenced for example by the slow-neutron fission of ²³⁵U and ²³⁹Pu. Furthermore the nucleus may tunnel through the barrier from its ground state. This process, called spontaneous fission, becomes more and more likely towards the end of the present Periodic Table. Extrapolation of the liquid-drop model beyond the known elements shows that the fission barrier should vanish in the region around element 114 leading to prompt fission. Only the action of nuclear shell closures will overcome this trend by keeping the nuclei spherical and stiff against deformation, producing a fission barrier which is now entirely due to shell effects¹⁷.

Most theoretical studies^{17,18} agree that the next shell closures beyond the existing elements should occur at proton number $Z = 114$ and neutron number $N = 184$. Hence a maximum

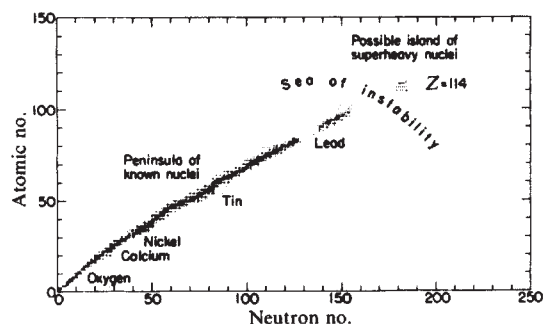


Fig. 1 Location of the island of superheavy elements relative to the peninsula of known nuclei. The heaviest points represent naturally occurring nuclei, the medium-weight points nuclei with half lives greater than 1 yr, and the lightest points nuclei with half lives less than 1 yr. The labelled elements correspond to nuclei with closed shells (from ref. 13).

the comparable magnitudes of neutron binding energies and fission barriers and by the need to extrapolate over a large, unexplored region. Most of the recent studies consider the production of superheavy elements in the r -process to be unlikely³⁹⁻⁴¹.

The large uncertainties in the predicted half lives as well as the open question of production in nucleogenesis have not discouraged experimentalists from examining hundreds of samples for superheavy elements, including lead from old cathedrals, manganese nodules from the ocean floor, water from hot springs, meteorites, and samples from the lunar surface. Extremely sensitive techniques were developed for such studies⁴², most of them being based on the direct or indirect detection of fission events. In counting untreated natural samples detection limits of 10^{-12} – 10^{-14} g of superheavy elements per g of sample can be achieved for superheavy nuclei of 10^9 yr half life. The most common methods are counting of fission fragment tracks produced by radiation damage in glass, mica or plastics, and counting of neutrons emitted in the fission process. The sensitivity of such methods can be increased by chemical enrichment and mass separation. In an extreme case⁴³ samples collected during the industrial processing of 10^3 – 10^4 tons of lead were further concentrated by chemical and mass separation, and a detection limit of 10^{-19} – 10^{-23} g per g was estimated for element 114.

Most of these searches produced negative results, but there are some observations which deserve attention and we report here on three: the hot spring water from the Cheleken peninsula, the Allende meteorite, and the Madagascan monazite.

In the hot springs from the Cheleken peninsula in the Caspian Sea a spontaneously fissioning activity was observed after passing about 2,000 m³ of water through 850 kg of an anion exchange resin⁴⁴. This particular spring water is known to be rich in volatile elements and is thought to collect material escaping from large depth in the Earth's mantle. Part of the resin was eluted with water and different acids followed by precipitation steps in the eluates. The samples were inspected with neutron-multiplicity counters which record the number of neutrons emitted in time coincidence. These neutrons are traced back to spontaneous fission events in the sample. The rates, about 0.5 fissions per day for a 9-kg sample of the resin and 5 fissions per day for the precipitates isolated from 170 kg of the resin, are much higher than what has been reported previously for any other sample inspected in low-background conditions. This allowed measurements of the neutron multiplicities with good statistics. Figure 4 compares the multiplicity distribution obtained⁴⁴ by summing the data for the resin and precipitate samples with the known distributions for several heavy nuclides and with some hypothetical distributions. Evidently the measurements rule out ²³⁸U but agree with heavy actinides such as ²⁵²Cf as a possible source of neutrons which may be present in the sample due to contamination in the laboratory or by fallout from nuclear weapons tests. From a small fraction of the combined precipitates, actinide elements were chemically isolated and inspected for α activity. Flerov *et al.*⁴⁴ argue that from these measurements, actinide nuclides except pure ²⁵²Cf can be excluded as the source of the neutrons. Further experiments seem to be necessary in order to identify the spontaneous fission activity in the Cheleken hot spring waters.

Evidence for now-extinct superheavy elements in a certain class of primitive meteorites was presented by Anders *et al.*^{45,46}. These carbonaceous chondrites contain xenon fractions with an excess of neutron-rich xenon isotopes. Such isotopes could stem from spontaneous or neutron-induced fission but the ¹³⁶Xe/¹³²Xe ratio observed is much larger than can be accounted for by any known fission reaction. The concentration of this fission-like xenon in different meteorites was observed to be correlated with the concentration of volatile elements such as thallium, bismuth and indium^{45,46}. Fission xenon plus volatile elements were found to be strongly enriched in a mineral fraction comprising less than 1% of the meteorite and consisting of carbon, chromite, spinel and an ill-defined mineral, probably

a sulphide^{47,48}. From the parallelism in the condensation history of the fission-like xenon and of volatile elements as inferred from such data, it was concluded that the neutron-rich xenon isotopes stem from spontaneous fission of a volatile superheavy element, most likely from element 115, eka-bismuth, but elements 114 and 113 are other possible candidates⁴⁸. Alternative explanations have been proposed mainly because the anomalous xenon fractions are also enriched in neutron-deficient isotopes^{49,50} and similar enrichments both of neutron-excess and neutron-deficient isotopes are also found for other elements⁵¹. It has also been pointed out⁵² that the predicted yields of xenon isotopes in spontaneous fission of superheavy nuclei would not fit the observed isotopic ratios. Whereas Anders *et al.* consider the progenitor of the fission-like xenon to be extinct, the Dubna group has reported weak spontaneous fission activities, about one event per month and kilogram, in several meteorites including Allende⁵³⁻⁵⁵. An attempt is being made to concentrate the activity from Allende by evaporation-condensation techniques to determine its atomic number⁵⁶.

Unexpectedly, evidence for element 126 and possibly 124 and 127 in nature was reported in 1976 by Gentry *et al.*²² who examined small monazite inclusions surrounded by giant haloes in Madagascan biotite. Radioactive haloes⁵⁷ are spherical zones of discolouration of the host mineral caused by radiation damage due to α particles emitted from the central inclusion. Cuts through such haloes show a circular ring structure corresponding to the ranges in the host mineral of the α particles from different members of the radioactive decay series descending from uranium and thorium deposited in the inclusion. In the giant haloes, however, the ranges correspond to α -particle energies around 14 MeV which are much higher than the energies of any known natural α -particle emitter but agree with predicted α -particle energies in the region of element 126. By bombarding the inclusions with a sharply collimated proton beam, characteristic X rays of the elements contained in the inclusions were excited and small peaks were seen at the expected positions of the $L\alpha_1$ X rays of elements 126, 124 and 127. The concentrations for these elements as estimated from the peak intensities were surprisingly high, about 10–100 p.p.m. However, the strongest peak attributed to element 126 could be accounted for by a nuclear γ ray from the ¹⁴⁰Ce(p, n)¹⁴⁰Pr reaction already occurring at the proton energy chosen⁵⁸, and other peaks could be assigned to K X rays of ordinary elements such as antimony and tellurium⁵⁹. The X ray spectra of the inclusions were remeasured^{60,61} with a different technique using a tunable monochromatic X-ray source for excitation, thus circumventing the interference by proton-induced nuclear reactions and by excitation of the mentioned ordinary elements

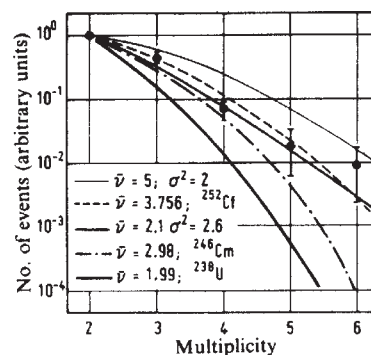


Fig. 4 Spontaneously fissioning activity in hot spring water from the Cheleken peninsula. Multiplicity distribution of fission neutrons emitted from samples obtained by ion-exchange and precipitation methods (●) compared with the measured multiplicity distribution for various actinide nuclides and with calculated distributions. The inset gives the corresponding values of the average number $\bar{\nu}$ of neutrons per fission and of its variance σ^2 (from ref. 44).

because of the differences in absorption edges. No evidence for superheavy element $L\alpha_1$ X rays was found⁶¹ at a concentration level about a factor of 100 below that previously reported²². Although Gentry⁶² agrees that the evidence was not confirmed, other members of the original group²² argue that problems in reproducing the earlier measurements are due to spatial inhomogeneities of the superheavy element distributions in the samples and to their volatility^{63,64}. Attempts to enrich superheavy elements from bulk monazites of the same origin by mass separation⁶⁵ and chemical separation⁶⁶ have failed. Surprisingly, evidence for high-energy α rays necessary to understand the giant haloes has recently been reported for the Madagascan monazite⁶⁷ in contrast with other negative findings^{68,69}. There seems to be no convincing evidence that giant haloes have anything to do with superheavy elements but none of the alternative explanations⁶² for the origin of these haloes has generally been accepted.

Searches at accelerators

Heavy-ion reactions seem to be the only practical way of producing superheavy elements in the laboratory. As shown in Fig. 1, one would jump in one step from the peninsula of known nuclei to the superheavy island. In heavy-ion reactions⁷⁰, three principal mechanisms play a part. The first is an exchange of a few nucleons between the two colliding nuclei in a rather distant collision and is called quasi-elastic transfer. The other two processes occurring in closer collisions are outlined⁷¹ in Fig. 5 with their characteristic time scales in units of 10^{-21} s. The upper branch illustrates complete fusion⁷² of the reacting nuclei to form a nearly spherical, excited compound nucleus which gains its energy from the fact that generally more kinetic energy is required to overcome the mutual electrostatic repulsion between the interacting nuclei than is consumed in the fusion process. The excitation energy is large enough to evaporate from the compound nucleus several neutrons, protons, or α particles followed by emission of γ rays to reach the ground state of the final evaporation residues which have the same or somewhat lower atomic number than the compound nucleus. For heavy compound nuclei, fission into two fragments of comparable size competes with evaporation and dominates for the heaviest known nuclei. Fusion to a compound nucleus occurs preferentially in reactions with relatively light projectiles such as carbon, oxygen or argon.

The third process⁷³⁻⁷⁵ is shown by the lower branch of Fig. 5. The two interacting nuclei stick together for a very short time, about 10^{-21} s, forming a composite system which separates again into two nuclei roughly identical with the interacting nuclei in the entrance channel. In spite of the short contact time, the kinetic energy of the projectile can be completely transformed into internal excitation and into rotation, and hence the process is called strongly damped or deep inelastic collision. During such a reaction a substantial number of protons and neutrons can be transferred between the interacting nuclei. The reaction

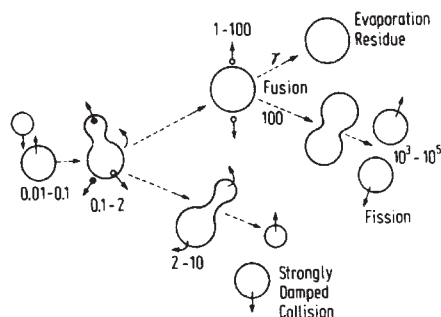


Fig. 5 Interactions between heavy nuclei leading to fusion into a compound nucleus (upper branch) and to damped collisions via a composite system (lower branch) with characteristic times in units of 10^{-21} s (after Brix⁷¹).

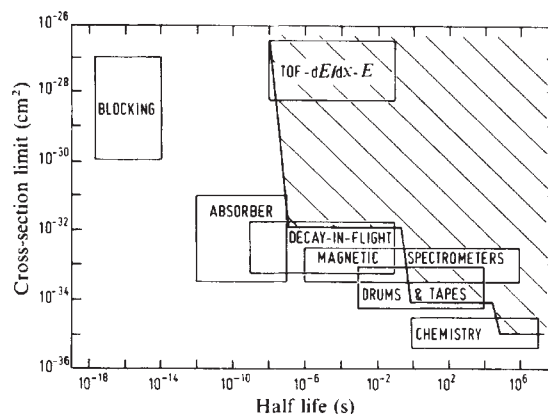


Fig. 6 Half-life region covered by various techniques applied to or applicable to the search for superheavy elements at accelerators, and upper limits for production cross-sections achievable with these techniques (from ref. 78).

products de-excite by emission of neutrons, protons, and γ rays and, in heavy systems, by fission. Although this novel type of nuclear reaction was discovered two decades ago^{76,77} it has received widespread interest only recently, after its important role in the interaction of heavy nuclei was recognised.

A wide variety of techniques has been developed and applied in searches for superheavy elements at accelerators⁷⁸. Figure 6 shows that techniques applicable to very short-lived nuclides are in general less sensitive than those suitable for longer half lives. Chemical methods combined with fission-fragment detection reach down to production cross-sections of 10^{-36} cm² corresponding to production rates of about one superheavy atom in 10^{12} nuclear collisions, but require half lives of at least a few seconds. Gas-jet systems for rapid transport of the activity from the production site to the detector system extend the half life range to shorter half lives by about one order of magnitude. Mechanical transport systems like tapes, drums or disks which are applicable to species of 10^{-3} s half life are even faster. The region down to 10^{-6} s is the domain of instruments based on the deflection of reaction products in magnetic and electric fields. Measurements of the time-of-flight (TOF) for a given distance and of the specific energy loss plus total energy ($dE/dx + E$) are widely used in studying heavy-ion reactions to obtain mass and atomic number distributions of the reaction products. When applied to superheavy nuclides they permit the detection of species with half lives down to 10^{-8} s. Detection of fission fragments emitted in flight from a superheavy nucleus passing a gas-filled tube extends the half life range which can be covered by one order of magnitude. By stopping superheavy atoms in a metal foil and observing their fission fragments in a direction perpendicular to the original flight path, nuclei with half lives down to 10^{-12} s can be detected. The extreme region, 10^{-14} – 10^{-18} s, becomes accessible by the blocking technique in which the reaction products are produced in a single crystal and the probability of escape of their fission fragments is measured for different crystallographic directions, indicating the distance traversed by the fissioning species in the crystal; thus the half life can be deduced since the velocity of the recoiling nucleus is known.

Because most of the heaviest synthetic elements were produced by heavy-ion fusion reactions it was quite natural to use fusion in attempts to synthesise superheavy elements. The problem with this approach lies in the extreme neutron excess of the most stable nuclei in the centre of the island which makes it impossible to reach those nuclei by any realistic fusion reaction. For example the first attempt^{10,12} to produce element 114

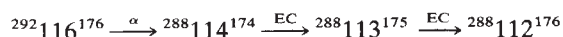


leads to a hypothetical compound nucleus with 174 neutrons only, which should further evaporate four neutrons due to its

excitation energy yielding the isotope $^{284}_{114}\text{X}^{170}$ far away from the $N = 184$ neutron shell. After detailed half life estimates for superheavy nuclei became available it seemed to be possible to overcome this problem by overshoot reactions, that is by producing nuclei beyond the region of highest stability but with rather high neutron number which should then decay by electron capture (EC) towards the centre of the island. To illustrate this a reaction is considered which has a crucial role in attempts to synthesise superheavy elements:



After evaporation of four neutrons the following decay chain would be expected, with half lives generally increasing along the chain¹⁹:



the latter nuclide should decay by spontaneous fission with a half life of ~ 1 h.

Table 1 surveys fusion reactions which have attempted to produce superheavy elements. The upper limit of the production cross-section is listed together with the half life range covered and an indication of whether chemical separation methods were applied. Table 1 re-emphasises the dilemma of fusion reactions: in the vicinity of element 114 the achievable neutron numbers are far below 184 whereas this closed neutron shell can only be reached with atomic numbers as high as 122. A more detailed analysis of these reactions in terms of excitation energy of the compound nuclei and survival against fission in the neutron evaporation chain can be found in a recent review⁹⁰.

Among the systems listed in Table 1 the reaction between ^{248}Cm and ^{48}Ca has been studied the most intensively since this reaction provides the closest approach to the island if both the proton and neutron numbers are considered. Experiments with this reaction are difficult, however, since neither the target, the artificial nuclide ^{248}Cm with 3.6×10^5 yr half life, nor the projectile ^{48}Ca , which has to be enriched from its low natural abundance of 0.19%, is generally available. The results reported so far for the ^{48}Ca -on- ^{248}Cm reaction are summarised in Fig. 7 where the upper limits for production cross-sections are plotted against the half life range covered with a particular technique. A variety of techniques has been applied. The Dubna group⁸¹ (D in Fig. 7) applied two different chemical separation methods and inspected the samples by spontaneous fission (SF) and α -particle counting (α). At Berkeley (BL) two different chemical pro-

Table 1 Attempts to synthesise superheavy elements by complete fusion reactions

System*	Compound nucleus	Upper limit for production cross-section‡ (cm ²)	Half life range covered	Ref.
$^{232}_{90}\text{Th} + ^{48}_{20}\text{Ca}$	$^{280}_{110}\text{X}^{170}$	3×10^{-35}	$\geq 3 \times 10^{-3}$ s	79
$^{231}_{91}\text{Pa} + ^{48}_{20}\text{Ca}$	$^{279}_{111}\text{X}^{168}$	4×10^{-35}	$\geq 3 \times 10^{-3}$ s†	79
$^{233}_{92}\text{U} + ^{48}_{20}\text{Ca}$	$^{281}_{112}\text{X}^{169}$	7×10^{-35}	≥ 2 h†	79
$^{248}_{96}\text{Cm} + ^{40}_{18}\text{Ar}$	$^{288}_{114}\text{X}^{174}$	2×10^{-32}	10^{-9} s–1 d	10, 80
$^{242}_{94}\text{Pr} + ^{48}_{20}\text{Ca}$	$^{290}_{114}\text{X}^{176}$	1×10^{-35}	2 h–1 yr†	81
$^{243}_{95}\text{Am} + ^{48}_{20}\text{Ca}$	$^{291}_{115}\text{X}^{176}$	2×10^{-35}	2 h–1 yr†	81
$^{246}_{96}\text{Cm} + ^{48}_{20}\text{Ca}$	$^{294}_{116}\text{X}^{178}$	2×10^{-35}	2 h–1 yr†	81
$^{248}_{96}\text{Cm} + ^{48}_{20}\text{Ca}$	$^{296}_{116}\text{X}^{180}$	See Fig. 7		
$^{208}_{82}\text{Pb} + ^{84}_{36}\text{Kr}$	$^{292}_{118}\text{X}^{174}$	1×10^{-30}	$\geq 6 \times 10^{-7}$ s	82
$^{208}_{82}\text{Pb} + ^{86}_{36}\text{Kr}$	$^{294}_{118}\text{X}^{176}$	6×10^{-35}	3×10^{-3} s–100 d	83
		1.5×10^{-30}	2×10^{-9} s–5 h	84
$^{238}_{92}\text{U} + ^{59}_{27}\text{Co}$	$^{297}_{119}\text{X}^{178}$	4×10^{-33}	1 s–30 h†	85
$^{238}_{92}\text{U} + ^{(60)}_{28}\text{Ni}$	$^{298}_{120}\text{X}^{178}$	2×10^{-33}	1 s–30 h†	85
$^{238}_{92}\text{U} + ^{(65)}_{29}\text{Cu}$	$^{303}_{121}\text{X}^{182}$	8×10^{-33}	1 s–30 h†	85
		2×10^{-33}	2 h–1 yr†	86
$^{238}_{92}\text{U} + ^{65}_{29}\text{Cu}$	$^{303}_{121}\text{X}^{182}$	4×10^{-34}	2×10^{-6} s–10 h	87
$^{232}_{90}\text{Th} + ^{76}_{32}\text{Ge}$	$^{308}_{122}\text{X}^{186}$	1×10^{-34}	5×10^{-3} s–1 yr	88
$^{136}_{54}\text{Xe} + ^{170}_{68}\text{Er}$	$^{306}_{122}\text{X}^{184}$	1.5×10^{-33}	2×10^{-6} s–10 h	87
$^{238}_{92}\text{U} + ^{76}_{32}\text{Ge}$	$^{314}_{124}\text{X}^{190}$	1×10^{-34}	5×10^{-3} s–1 yr	88
$^{243}_{95}\text{Am} + ^{68}_{30}\text{Zn}$	$^{311}_{125}\text{X}^{186}$	5×10^{-32}	10^{-9} s–5 d	89
$^{232}_{90}\text{Th} + ^{84}_{36}\text{Kr}$	$^{316}_{126}\text{X}^{190}$	5×10^{-30}	$\geq 6 \times 10^{-7}$ s	82
$^{238}_{92}\text{U} + ^{84}_{36}\text{Kr}$	$^{322}_{128}\text{X}^{194}$	8×10^{-29}	$\geq 6 \times 10^{-7}$ s	82

* If the projectile beam was not isotopically clean the mass number used here is in parentheses.

† Denotes studies in which chemical separation methods were applied at least in part of the measurements.

‡ Note that in calculating cross-section limits different authors use different assumptions about the width of the excitation function for the nuclear reaction involved; this introduces an uncertainty of about one order of magnitude⁸⁶.

cedures (chem) were used⁹¹ and an experiment designed to detect extremely volatile elements during and after bombardment was performed (gas)⁹². Attempts were also made to detect short-lived species by counting without chemical treatment the catcher foils in which the reaction products recoiling out of the target were collected (foils)⁹¹. In further experiments, the recoil atoms were stopped in a gas and transported with a gas jet to a rotating-wheel counting system (VW)⁹³. Finally spontaneous-fission decay in flight was searched for (dif)⁹³.

Why these experiments have failed has been discussed in detail by Seaborg *et al.*⁹⁰. The bombardments were carried out at an average energy exceeding the coulomb barrier by a factor of 1.09 resulting in some 40 MeV of excitation energy of the compound nucleus. Based on the measured production cross-section of the fusion product $^{254}_{102}\text{No}$ in the $^{48}\text{Ca} + ^{208}\text{Pb}$ reaction, a fusion cross-section of at least 10^{-27} cm² was estimated for the ^{48}Ca - ^{248}Cm system. Thus the reasons for negative results were sought in the evaporation chain. Using two sets of calculated fission barriers the overall survival probability was estimated. Whereas the optimistic set¹⁹ predicts that 96% of the compound nuclei should arrive at ^{292}X , the more pessimistic set²¹ predicts less than $4 \times 10^{-9}\%$, corresponding to a production cross-section of the order of 10^{-38} cm². One may attempt to increase the survival probability by lowering the bombarding energy in order to produce less excited compound nuclei. In theoretical studies^{94,95} of fusion reactions it is pointed out, however, that the bombarding energy in the performed experiments was still too low⁹⁵ by 10–30 MeV since an extra push of energy is required to fuse two nuclei. A general consequence of this analysis⁹⁰ is that it

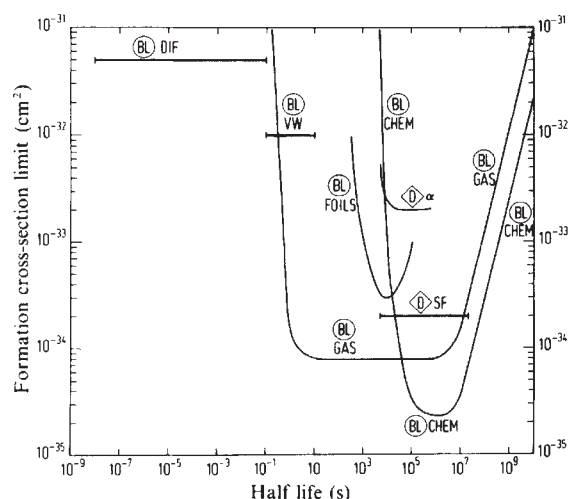


Fig. 7 Upper limits of the production cross-section for superheavy elements in the reaction of ^{248}Cu with ^{48}Ca versus the half-life range covered with different techniques. The data are from work of the Berkeley–Livermore group^{91–93} (BL) and the Dubna group⁸¹ (D); for details see text.

may indicate a steeper descent of the island on its west shore than found in calculations as shown by Fig. 2. If this is the case, to produce superheavy elements by complete fusion is apparently hopeless, a result which agrees with the numerous negative results listed in Table 1. Oganessian *et al.*⁸¹ argue that either a low fusion cross-section or short half lives may explain the negative results with the $^{48}\text{Ca} + ^{248}\text{Cm}$ reaction.

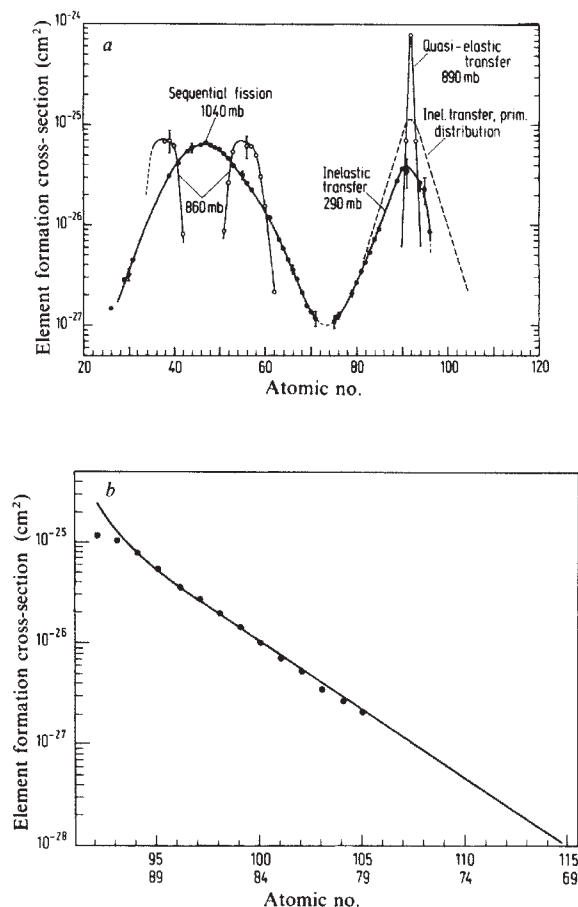
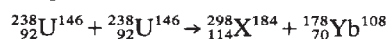


Fig. 8 *a*, Element formation cross-sections in the reaction of ^{238}U with ^{238}U as obtained by a radiochemical analysis of the reaction products produced in thick uranium targets bombarded with 7.5 MeV AMU^{-1} uranium ions (from ref. 102). *b*, Element formation cross-sections for complementary fragments in the $^{238}\text{U} + ^{238}\text{U}$ reaction deduced from experimental data as shown in *a* (●), compared with values calculated (solid line) using the diffusion model of damped collisions (after Riedel and Nörenberg¹⁰³).

Are there any alternatives? A first hint in other directions seemed to be the observation of a 150-d spontaneously fissioning activity^{96,97} produced with a cross-section of 10^{-33} cm^2 in bombardments of ^{238}U with ^{136}Xe and attributed to a super-heavy element because of its chemical behaviour which was tested in a few bombardments. Flerov *et al.*^{96,97} assumed that a fusion-fission process occurred but this is an unlikely mechanism for reactions between very heavy nuclei, as more recent studies have shown. Attempts to reproduce these findings, failed^{85,98-100}, however, in spite of an increase of the sensitivity by two orders of magnitude⁹⁹.

An alternative pathway to the superheavy elements was opened^{101,102,35} by the first studies of the reaction between two colliding uranium nuclei carried out at the Darmstadt UNILAC accelerator when, for the first time, uranium beams with energies sufficient to overcome the interaction barrier even for uranium became available. These studies^{101,102} demonstrated that the superheavy island might be reachable by exchange of nucleons in damped collisions. The evidence is still indirect and

is based on the observation of complementary products. One of the colliding uranium nuclei increases its proton and neutron number at the expense of the other uranium nucleus



This implies, of course, that such damped collisions are binary reactions and that charged-particle evaporation plays only a minor part. There is strong evidence for these assumptions⁷³⁻⁷⁵

Figure 8*a* shows the element distribution in the reaction between two ^{238}U nuclei as obtained in radiochemical studies¹⁰² by measuring a network of formation cross-sections for individual nuclides. The element yields peak at uranium in a narrow distribution due to quasi-elastic transfer. The underlying, broader distribution results from nucleon transfer in damped collisions. Reaction products from both types of reactions undergo fission as is evident from the broad distribution of fission products between element 30 and 70 and the steep decrease of the cross-sections beyond uranium due to increasing fissionability of transuranium nuclei. This decrease was followed over eight orders of magnitude up to fermium ($Z = 100$) whose element formation cross-section is $8 \times 10^{-33} \text{ cm}^2$. Before fission occurs the element distributions both in the quasi-elastic and in the damped collisions should be symmetric around uranium for reasons already mentioned. The primary distribution originating from damped collisions can easily be reconstructed and is shown by the dashed curve in Fig. 8*a*. Extrapolation of this distribution to element 70 results in a cross-section of 10^{-28} cm^2 for the element 114-70 combination. A more sophisticated extrapolation is shown in Fig. 8*b* where the element cross-sections obtained experimentally are compared with values calculated¹⁰³ using a model which treats the nucleon transfer in damped collisions as a diffusion process¹⁰⁴. The agreement between experimental and calculated cross-sections is very satisfying. For the element 114-70 combination again a production cross-section of 10^{-28} cm^2 is calculated.

But what fraction of element 114 may survive fission? It is an attractive feature of damped collisions that the reaction products are formed with very broad distributions in excitation energy and angular momentum, tailing to low values which are essential for survival. Counter experiments¹⁰¹ and an analysis of production cross-sections of the highly fissionable transcurium nuclides¹⁰² have shown that the excitation energies of fragments originating from the uranium-on-uranium reaction are unusually low if compared with other reactions between heavy nuclei. Calculations¹⁰³ with the diffusion model predict at 8.3 MeV AMU^{-1} bombarding energy production cross-sections of about 10^{-34} cm^2 for element 114 nuclei with <30 MeV excitation energy, an energy range for which the survival probability is estimated²⁵ to be >50% for the first neutron-evaporation step provided the neutron number is close to 184. It has been shown for damped collisions with light^{75,105} and heavy¹⁰⁶ projectiles that the most probable neutron numbers for a given element split corresponds to a maximum mass difference between interacting and product nuclei with a minor correction for nuclear pairing energies. Applying this concept to the uranium-on-uranium reaction leads to $N = 180$ and $N = 182$ as the most probable neutron numbers for element 114 fragments. Taking all these estimates together one may expect production cross-sections of about 10^{-35} cm^2 for element 114. This corresponds, with the accessible beam intensities, to production rates of a few atoms per day or per week a rate just exceeding the detection limit of the most sensitive methods available at present.

Direct searches for superheavy elements in the uranium-on-uranium reaction have so far been negative. Looking for spontaneous fission events from reaction products implanted in a semiconductor detector gave an upper cross-section limit of $2 \times 10^{-32} \text{ cm}^2$ for half lives between milliseconds and months¹⁰¹. With the gas-jet technique an upper limit of $1.5-3 \times 10^{-33} \text{ cm}^2$ has been reported for half lives between 1 and 10^5 s ⁸⁵, and 10 and 10^3 s ¹⁰⁷. A more sensitive chemical experiment with a separation method based on volatilisation gave an upper limit of

10^{-35} cm² for half lives between several hours and one year, but further experiments using modified techniques are underway¹⁰⁸.

The use of heavier elements is obviously very attractive. Calculations with the diffusion model predict¹⁰³ an increase in the production cross-section for element 114 by two orders of magnitude when ²⁴⁸Cm instead of uranium is bombarded with uranium projectiles. On the other hand the use of ²⁴⁴Pu as projectile as proposed by Seaborg *et al.*⁹⁰ offers no advantages if compared with uranium¹⁰³. Another factor of seven may be gained with a ²⁴⁹Cf target but the gain may be illusory because of the limited availability of such exotic targets. Furthermore the estimated most probable neutron number moves away from the 184 neutron shell for targets heavier than ²⁴⁴Pu.

Another mechanism for formation of superheavy elements has been suggested¹⁰⁹. When fissile nuclei are used as target or projectile they may undergo fission under the influence of the other partner, and one of the fission fragments may fuse with the projectile to form a superheavy nucleus. Experiments¹¹⁰ follow-

ing this suggestion in which lead was bombarded with ¹³⁶Xe yielded negative results with an upper cross-section limit of 1.2×10^{-30} cm² for half lives between 10^{-12} and 10^4 s.

Outlook

There is no convincing evidence that superheavy elements have been discovered. On the contrary, the negative results produced by strong efforts in many laboratories during the past dozen years are discouraging. Yet, there is at least one natural source, the hot spring water from the Cheleken peninsula, which deserves attention, and similar springs may exist elsewhere. Also, reactions between very heavy nuclei have not yet been fully explored as a possible pathway to the superheavy elements in the laboratory. Whatever the final outcome, the intense exploration occasioned by the search for superheavy elements is bound to lead to a better understanding of the forces that terminate the Periodic Table at its upper end.

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- Wheeler, J. A. *Proc. Int. Conf. Peaceful Uses Atomic Energy* Vol. 2, 155 (United Nations, New York, 1966).
- Scharff-Goldhaber, G. *Nucleonics* **15**, 122 (1957).
- Werner, F. G. & Wheeler, J. A. *Phys. Rev.* **109**, 126 (1958).
- Myers, W. D. & Swiatecki, W. J. *Nucl. Phys.* **81**, 1 (1966).
- Meldner, H. *Arkiv Fysik* **36**, 593 (1967).
- Nilsson, S. G. *et al. Nucl. Phys.* **A115**, 545 (1968).
- Nilsson, S. G. *et al. Nucl. Phys.* **A131**, 1 (1969).
- Mosel, U. & Greiner, W. *Z. Physik* **222**, 261 (1969).
- Thompson, S. G., Gatti, R. C., Moretto, L. G., Bowman, H. R. & Michel, M. C. *Univ. California Radiation Lab. Berkeley Rep. UCRL-18667*, 277 (1969).
- Nilsson, S. G., Thompson, S. G. & Tsang, G. F. *Phys. Lett.* **28B**, 458 (1969).
- Flerov, G. N. & Perlygin, V. P. *Atomn. Energiya* **26**, 520 (1969); *Sov. J. Atom. En.* **26**, 603 (1969).
- Bowman, H. R. *et al. Univ. California Radiation Lab. Berkeley Rep. UCRL-17989*, 147 (1968).
- Bemis, C. E. & Nix, J. R. *Comm. Nucl. Part. Phys.* **7**, 65 (1977).
- Herrmann, G. *Int. Rev. Sci. Inorg. Chem.*, Ser. 2, Vol. 8, (ed. Maddock, A. G.) 221 (Butterworth, London, 1975).
- Nilsson, S. G. & Nilsson, N. R. (eds) *Phys. Scr.* **10A** (1974).
- Lodhi, M. A. K. (ed.) *Proc. int. Symp. on Superheavy Elements* (Pergamon, New York, 1978).
- Nix, J. R. *A. Rev. nucl. Sci.* **22**, 65 (1972).
- Nilsson, S. G. in *Proc. int. Symp. on Superheavy Elements* (ed. Lodhi, M. A. K.) 237 (Pergamon, New York, 1978).
- Fiset, E. O. & Nix, J. R. *Nucl. Phys.* **A193**, 647 (1972).
- Sobiczewski, A. *Proc. int. Symp. on Superheavy Elements* (ed. Lodhi, M. A. K.) 274 (Pergamon, New York, 1978).
- Randrup, J., Larsson, D. E., Möller, P., Sobiczewski, A. & Zukasiak, A. *Phys. Scr.* **10A**, 60 (1974).
- Gentry, R. V. *et al. Phys. Rev. Lett.* **37**, 11 (1976).
- Brack, M., Quentin, P. & Vautherin, D. in *Proc. int. Symp. on Superheavy Elements* (ed. Lodhi, M. A. K.) 309 (Pergamon, New York, 1978).
- Kolb, D. Z. *Phys. A280*, 143 (1977).
- Moretto, L. G. *Nucl. Phys.* **A180**, 337 (1972).
- Mustafa, M. G. in *Proc. int. Symp. on Superheavy Elements* (ed. Lodhi, M. A. K.) 284 (Pergamon, New York, 1978).
- Nix, J. R. *Phys. Lett.* **30B**, 1 (1969).
- Schmitt, H. W. & Mosel, U. *Nucl. Phys.* **A186**, 1 (1972).
- Hoffman, D. C. in *Proc. int. Symp. on Superheavy Elements* (ed. Lodhi, M. A. K.) 89 (Pergamon, New York, 1978).
- Fricke, B. *Structure Bonding* **21**, 89 (1975).
- Keller, O. L. & Seaborg, G. T. *A. Rev. nucl. Sci.* **27**, 139 (1977).
- Eichler, B. J. *Kernenergie* **19**, 307 (1976) **35**, 4001 (1973).
- Pitzer, K. S. *J. chem. Phys.* **63**, 1032 (1975).
- Kratz, J. V., Liljenzin, J. O. & Seaborg, G. T. *Inorg. Nucl. Chem. Lett.* **10**, 951 (1974).
- Herrmann, G. in *Proc. int. Symp. on Superheavy Elements* (ed. Lodhi, M. A. K.) 24 (Pergamon, New York, 1978).
- Carlson, T. A. & Nestor, C. W. *Atomic Nucl. Data Tables* **19**, 153 (1977).
- Scofield, J. H. *Atomic Nucl. Data Tables* **14**, 121 (1974).
- Harvey, B. G. *et al. Science* **193**, 1271 (1976).
- Schramm, D. N. & Fiset, E. O. *Astrophys. J.* **180**, 551 (1973).
- Howard, W. M. & Nix, J. R. *Nature* **247**, 17 (1974).
- Mathews, G. J. & Viola, V. E. *Nature* **261**, 382 (1976).
- Herrmann, G. in *Phys. Scr.* **10A**, 71 (1974).
- McMinn, J., Ihle, H. R. & Wagner, R. *Nucl. Instrum. Meth.* **139**, 175 (1976).
- Flerov, G. N. *et al. Z. Phys.* (submitted).
- Anders, E. & Heymann, D. *Science* **164**, 821 (1969).
- Anders, E. & Larimer, J. W. *Science* **175**, 981 (1972).
- Lewis, R. S., Srinivasan, B. & Anders, E. *Science* **190**, 1251 (1975).
- Anders, E., Higuchi, H., Gros, J., Takahashi, H. & Morgan, J. W. *Science* **190**, 1262 (1975).
- Manuel, O. K. & Sabu, D. D. *Science* **195**, 208 (1977).
- Frick, U. & Moniot, R. K. *8th Lunar Sci. Conf.*, 229 (1977).
- Ballad, R. V., Oliver, L. L., Downing, R. G. & Manuel, O. K. *Nature* **277**, 615 (1979).
- Steinberg, E. P. & Wilkins, B. D. *Astrophys. J.* **223**, 1000 (1978).
- Popeko, A. G., Skobelev, N. K., Ter-Akopyan, G. M. & Goncharov, G. N. *Phys. Lett.* **52B**, 417 (1974).
- Flerov, G. N., Ter-Akopyan, G. M., Popeko, A. G., Fefilov, B. V. & Subbotin, V. G. *Yad. Fiz.* **26**, 449 (1977); *Sov. J. nucl. Phys.* **26**, 237 (1977).
- Popeko, A. G. & Ter-Akopyan, G. M. *Yad. Fiz.* **29**, 604 (1979).
- Zvara, I. *et al. Yad. Fiz.* **26**, 455 (1977); *Sov. J. Nucl. Phys.* **26**, 240 (1977).
- Gentry, R. V. *A. Rev. nucl. Sci.* **23**, 347 (1973).
- Bosch, F. *et al. Phys. Rev. Lett.* **37**, 1515 (1976); *Z. Phys.* **A280**, 39 (1977).
- Wölfl, W. *et al. J. Phys.* **G3**, L33 (1977).
- Sparks, C. J., Raman, S., Yakel, H. L., Gentry, R. V. & Krause, M. O. *Phys. Rev. Lett.* **38**, 205, 617 (1977).
- Sparks, C. J., Raman, S., Ricci, E., Gentry, R. V. & Krause, M. O. *Phys. Rev. Lett.* **40**, 507 (1978).
- Gentry, R. V. in *Proc. int. Symp. on Superheavy Elements* (ed. Lodhi, M. A. K.) 123 (Pergamon, New York, 1978).
- Cahill, T. A., *et al. Phys. Rev.* **C17**, 1183 (1978).
- Cookson, J. A., Fletcher, N. R., Kemper, K. W., Medsker, L. R. & Cahill, T. A. in *Proc. int. Symp. on Superheavy Elements* (ed. Lodhi, M. A. K.) 164 (Pergamon, New York, 1978).
- Stephan, C., Ephre, M., Cieślak, E., Sowiński, M. & Tys, J. *Phys. Rev. Lett.* **37**, 1534 (1976).
- Stakemann, R., Heimann, R., Herrmann, G., Tittel, G. & Trautmann, N. (to be published).
- Chevallier, A., Chevallier, J., Pape, A., Debeauvais, M. & Leroux, B. in *Proc. int. Symp. on Superheavy Elements* (ed. Lodhi, M. A. K.) 155 (Pergamon, New York, 1978).
- Brandt, R. in *Proc. int. Symp. on Superheavy Elements* (ed. Lodhi, M. A. K.) 103 (Pergamon, New York, 1978).
- Fireman, E. L. in *Proc. int. Symp. on Superheavy Elements* (ed. Lodhi, M. A. K.) 172 (Pergamon, New York, 1978).
- Hodgson, P. E. *Nuclear Heavy-Ion Reactions* (Clarendon, Oxford, 1978).
- Brix, P. *Max-Planck-Institut für Kernphysik Heidelberg Rep. MPI H-1977-V41*.
- Lefort, M. *Rep. Progr. Phys.* **39**, 129 (1976).
- Schröder, W. U. & Huizenga, J. R. *A. Rev. nucl. Sci.* **27**, 465 (1977).
- Lefort, M. & Ngô, C. *Ann. Phys.* **3**, 5 (1978).
- Volkov, V. V. *Phys. Rep.* **44**, 93 (1978).
- Kaufmann, R. & Wolfgang, R. *Phys. Rev. Lett.* **3**, 232 (1959).
- Kaufmann, R. & Wolfgang, R. *Phys. Rev.* **121**, 192 (1961).
- Nitschke, J. M. in *Proc. int. Symp. on Superheavy Elements* (ed. Lodhi, M. A. K.) 42 (Pergamon, New York, 1978).
- Ter-Akopyan, G. M. *et al. Yad. Fiz.* **29**, 608 (1979).
- Thompson, S. G. *et al. Univ. of California Radiation Lab. Berkeley Rep. UCRL 18667* (1969) 283.
- Oganessian, Yu. Ts., *et al. Nucl. Phys.* **A294**, 213 (1978).
- Colombani, P. *et al. Phys. Lett.* **42B**, 208 (1972).
- Wirth, G. *et al. Ges. für Schwerionenforschung Darmstadt A. Rep.* 1976, 72.
- Butler, P. A. *et al. Phys. Lett.* **74B**, 222 (1978).
- Aumann, D. C., Faleschini, H., Friedmann, L. & Weismann, D. *Phys. Lett.* **82B**, 361 (1979).
- Esterlund, R. A. *et al. Phys. Rev.* **C15**, 319 (1977).
- Münzenberg, G. *et al. Ges. für Schwerionenforschung Darmstadt A. Rep.* 1977, 75.
- Flerov, G. N. *et al. Yad. Fiz.* **19**, 492 (1974); *Sov. J. nucl. Phys.* **19**, 247 (1974).
- Pleve, A. A., Demin, A. G., Kusch, V., Miller, M. B. & Danilov, N. A. *Yad. Fiz.* **19**, 252 (1974); *Sov. J. nucl. Phys.* **19**, 123 (1974).
- Seaborg, G. T., Loveland, W. & Morrissey, D. J. *Science* **203**, 711 (1979).
- Otto, R. J. *et al. J. inorg. nucl. Chem.* **40**, 589 (1978).
- Illige, J. D. *et al. Phys. Lett.* **78B**, 209 (1978).
- Ghiorso, A. *et al. Lawrence Berkeley Lab. Rep. LBL 6575*, 242 (1977).
- Nix, J. R. & Sierk, A. R. *Phys. Rev.* **C15**, 2072 (1977).
- Sierk, A. R. in *Proc. int. Symp. on Superheavy Elements* (ed. Lodhi, M. A. K.) 479 (Pergamon, New York, 1978).
- Flerov, G. N. & Oganessian, Yu. Ts. in *Proc. Symp. Chemistry of Transuranic Elements*, Moscow (1972).
- Flerov, G. N. *Proc. Int. Conf. on Reactions between Complex Nuclei* (eds Robinson, R. L., McGowan, F. K., Ball, J. B. & Hamilton, J. H.) Vol. 2, 459 (North-Holland, Amsterdam, 1974).
- Otto, R. J., Ghiorso, A., Lee, D., Leber, R. E., Yashita, S. & Seaborg, G. T. *Radiochim. Acta* **24**, 3 (1977).
- Gäggeler, H. *et al. Z. Phys.* **286**, 419 (1978).
- Becker, H. J., Lund, T., Brandt, R., Molzahn, D. & Jungclas, H. *Ges. für Schwerionenforschung Darmstadt A. Rep.* 1977, 24.
- Hildenbrand, K. D. *et al. Phys. Rev. Lett.* **39**, 1065 (1977).
- Schädel, M. *et al. Phys. Rev. Lett.* **41**, 469 (1978).
- Riedel, C. & Nörenberg, W. *Z. Phys.* **A290**, 385 (1979).
- Nörenberg, W. *J. Phys.* **37**, C5, 141 (1976).
- Volkov, V. V. *Proc. Int. Conf. on Reactions between Complex Nuclei* Vol. 2 (eds Robinson, R. L., McGowan, F. K., Ball, J. B. & Hamilton, J. H.) 363 (North-Holland, Amsterdam, 1974).
- Kratz, J. V. *et al. Ges. für Schwerionenforschung Darmstadt A. Rep.* 1978.
- Jungclas, H. *et al. Phys. Lett.* **79B**, 58 (1978).
- Trautmann, N. *et al. Ges. für Schwerionenforschung Darmstadt A. Rep.* 1978.
- Deubler, H. H., Lekkas, K., Sperr, P. & Dietrich, K. *Z. Phys.* **A284**, 237 (1978).
- Sperr, P. *et al. Z. Phys.* **A287**, 57 (1978).

articles

Boninites, komatiites and ophiolitic basalts

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The rare Phanerozoic rock type boninite petrographically resembles Archaean basaltic komatiites and the range of boninite compositions overlaps that of basaltic komatiites. Quench amphibole and hydrous glass in the groundmass of boninites confirm earlier ideas that they may be the products of partial melting of peridotite in hydrous conditions, whereas basaltic komatiites have been assumed to result from fractionation of dry melts. Where field relationships are known, boninites are found either in ophiolites or a fore-arc tectonic environment.

BONINITES, first described in 1890 (ref. 1) in Cenozoic lavas from the Western Pacific, have chemical compositions similar in some respects to magnesian andesites. They bear a remarkable resemblance to basaltic komatiites which form a substantial component of Archaean volcanism (Fig. 1). Rocks which we consider to be boninites, containing rapidly grown pyroxene and microphenocrysts of olivine and/or low-calcium pyroxene set in a glassy matrix are relatively widespread amongst ophiolitic basalts. This article offers new petrological information on these important rocks with implications for the origin of at least some ophiolites.

The use of the term boninite requires explanation. Kikuchi¹ described two rocks from Chichijima in the Bonin or Ogasawara group, Western Pacific. One contained phenocrysts of orthopyroxene (bronzite) and skeletal augite set in a dark glassy matrix "comparatively free of the palagonitic alteration product". The chemical analysis (analysis 1, Table 1) was of a "glassy portion" of the rock which we interpret as meaning close to the liquid composition. The other rock had phenocrysts of bronzite, augite and plagioclase feldspar in a groundmass of augite, plagioclase and glass (analysis 2). Petersen² introduced the name boninite and presented a third analysis of a soda-rich, probably altered rock (analysis 3) which he described as a feldspar-free, glass-rich variety of bronzite-andesite with olivine and augite.

Later, Tröger³ gave analysis 1 as the type boninite and mentioned possible groundmass olivine as part of the mode. We concur with Tröger's choice and redefine boninite as follows: a highly magnesian but relatively siliceous, glassy rock containing one or more varieties of pyroxene, some or all of which have a morphology characteristic of rapid growth, accessory magnesiochromite and, commonly, minor amounts of olivine. Laths of amphibole or plagioclase microlites are rare.

Although boninites could be called high-magnesium andesites⁴ their mineralogy, texture and high chromium and nickel contents differ so markedly from those of typical andesites that we consider the separate term justified.

Samples conforming to our definition of boninite were studied from the type locality, Chichijima in the Bonin islands^{1,2,5,6} (Oligocene), the Dabi volcanics, Cape Vogel peninsula, eastern Papua⁷⁻⁹ (?Oligocene), the uppermost Upper Pillow lavas of the Troodos massif^{10,11} and Arakapas Fault belt¹², Cyprus (Cretaceous), the Agrilia Formation of the Othris Mountains, Central Greece¹³ (Triassic) and from dredge haul 1403 at 12° N on the inner wall of the Mariana Trench^{14,15} (Upper Eocene, unpublished data). Boninite has recently been recovered from a similar tectonic environment at 18° N in Hole 458 on Leg 60 of the IPOD drilling programme¹⁶. Palaeozoic rocks with similar

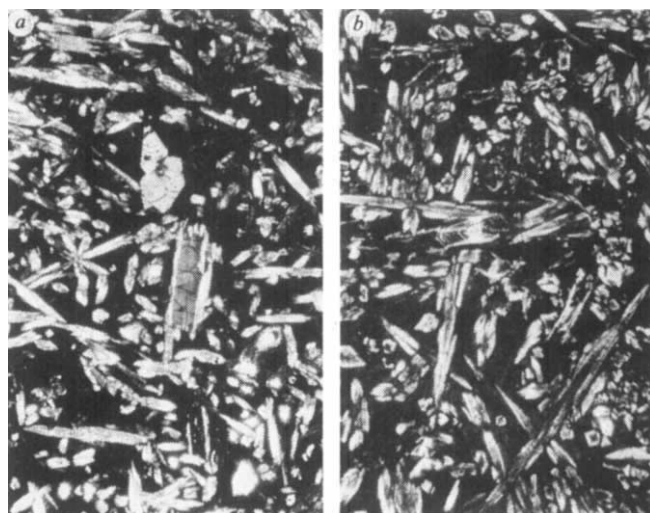


Fig. 1 Comparison of textures of boninite and basaltic komatiite. *a*, Boninite, specimen 29473, Upper Pillow Lavas, Cyprus. Microphenocrysts of olivine, orthopyroxene jacketed by augite. Groundmass pyroxenes are mainly augites, but a few are zoned from pigeonite to augite. 1.5 mm wide, crossed polars. *b*, Basaltic komatiite NG137 from a 2-m thick flow (Fig. 2, ref. 22), Belingwe greenstone belt, Rhodesia. Skeletal olivines and groundmass augite surrounding cores of pigeonite, now altered. 2.5 mm wide, plane polarised light.

Table 1 Chemical compositions of boninites and basaltic komatiites

	1 ¹	Original boninites Bonin Islands 2 ¹	3 ²	Inner wall Mariana Trench, 12°N ¹⁵ 1403-45	Dabi volcanics C. Vogel, Papua ⁹ LB105	Upper Pillow Lavas, Cyprus 29473	Arakapas Fault Belt, Cyprus ¹² 1303	Belingwe Greenstone Belt, Rhodesia ²¹ NG137	Munro Township, Northern Ontario Canada ²³ P9-185
SiO ₂	54.4	53.2	53.9	55.9	54.1	51.4	52.2	50.9	47.2
TiO ₂	ND	ND	ND	0.21	0.30	0.36	0.20	0.52	0.43
Al ₂ O ₃	12.9	16.2	18.0	10.8	9.5	12.1	12.1	10.3	11.9
Fe ₂ O ₃	7.1	10.3	ND	2.9	2.5	1.8	2.4	1.3	1.8
FeO	ND	ND	4.9	6.0	6.5	5.6	6.5	10.0	9.5
MnO	ND	ND	ND	0.18	0.15	0.14	0.17	0.16	0.20
MgO	12.7	6.7	4.6	11.8	13.0	10.5	12.1	12.8	12.6
CaO	5.1	10.1	7.6	6.0	5.5	9.8	10.0	8.6	9.9
Na ₂ O	2.1	1.8	3.9	1.8	0.75	1.4	0.70	2.0	1.9
K ₂ O	0.35	0.35	1.1	0.7	0.41	0.27	0.25	0.23	0.01
P ₂ O ₅	ND	ND	ND	0.03	0.07	0.03	0.04	0.03	ND
H ₂ O	5.5	1.6	4.6	4.2	7.4	5.7	3.5	3.5	3.9
CO ₂	ND	ND	ND	0.0	0.14	0.30	ND	ND	0.02
Cr ₂ O ₃	ND	ND	ND	0.15	0.12	0.09	0.12	0.20	ND
NiO	ND	ND	ND	0.03	0.03	0.03	0.05	0.03	ND
Total	100.1	100.2	98.6	100.7	100.4	100.3	100.3	100.5	99.3

ND, not determined.

textures occur in the Betts Cove ophiolite¹⁷ and within the Central Heathcote 'greenstone' belt, Victoria¹⁸, but these contain augite and chromite as the only relict phases. Probable equivalents from Rambler, Newfoundland¹⁹, and the Dundas Trough, Tasmania²⁰, have been completely recrystallised.

Basaltic komatiites with good textural and mineralogical preservation are rare; our analytical study involved specimens from the Belingwe greenstone belt Zimbabwe-Rhodesia^{21,22}, and was supplemented by data from equally fresh material in the Abitibi belt of northern Ontario^{23,24}. Texturally, basaltic komatiite flows contain clinopyroxene, some or all with a morphology indicative of rapid growth; olivine, in the more magnesian lavas; and commonly groundmass plagioclase which varies from microlitic to intergranular. Pigeonite cores to augite are relatively rare and orthopyroxene is restricted to the cumulus zones of very thick flows.

Mineralogy

The compositional ranges of the primary minerals are given in Table 2; the complete analyses will be presented elsewhere. Olivine compositions in the boninites were found to vary from Fo₉₃₋₈₃, depending largely on crystal size. Phenocrysts (generally 1.0–10 mm long) found in the 'ultramafic lavas' of Cyprus^{25,26} and Othris are Fo₉₃₋₉₁. Microphenocrysts (0.2–0.6 mm) form 5–10% of the mode in pseudomorph or unaltered form in almost all the other samples studied; the vast majority have compositions between Fo₉₀ and Fo₈₇. Tiny skeletal olivines occur in the glassy matrix of several of the Cyprus boninites and have compositions between Fo₈₈ and Fo₈₃. Some of the phenocrysts but few of the microphenocrysts are corroded and none shows a reaction relationship to calcium-poor pyroxene.

In basaltic komatiites, olivine (almost invariably pseudomorphed) typically occurs as microphenocrysts or skeletal grains 0.2–0.4 mm long. One rock from Belingwe, Zimbabwe-Rhodesia, has completely unaltered olivine microphenocrysts of composition Fo₈₈ and abundant groundmass olivine, sometimes euhedral, with compositions as iron-rich as Fo₈₂. In a thick basaltic komatiite flow (19% MgO) from Munro Township cumulus olivine of comparable composition (Fo₈₉₋₈₇) was found²⁷. Olivines in both the Belingwe komatiite and boninites generally have high nickel and calcium contents (>0.20%) but boninitic olivines have lower chromium content (<0.08%), suggesting that the order of crystallisation we observe petrographically in komatiites, olivine → chromite, is reversed in boninites.

Calcium-poor pyroxene occurs in all boninites except those from Othris, either as clinoenstatite (Cape Vogel, Mariana Trench) and orthopyroxene, or exclusively the latter. Phenocrysts (up to 3 mm) are present in most of the Cape Vogel rocks and in a few of the Mariana Trench and Bonin samples. Their Al₂O₃ contents are low, 0.1–1.1%, although they have very high Mg/(Mg + Fe) ratios (Fig. 2, open symbols). Microphenocrysts of orthopyroxene are common in the Cyprus rocks and have 'quench' overgrowths of augite. Groundmass orthopyroxene consists of laths intergrown with pigeonite and augite, or discrete cruciform shapes. Magnesian pigeonite has either nucleated on orthopyroxene grains of slightly higher Mg/(Mg + Fe) ratio or from the liquid. More often augite mantles orthopyroxene which may in turn be intergrown with clinoenstatite. Phenocrysts of calcic pyroxene are rare both in boninites and komatiites; their compositions lie close to En₅₂Wo₄₁Fs₇.

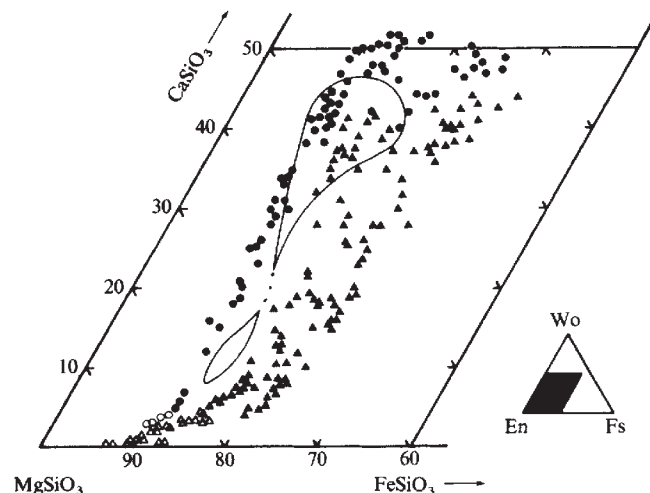


Fig. 2 Compositional variation of calcium-poor (open symbols) and groundmass calcic pyroxenes (closed symbols) within portion of the system MgSiO₃-FeSiO₃-CaSiO₃. All Fe is expressed as Fe²⁺. Origin of specimens: ○●, Cyprus and Othris; △▲, Bonin Island, C. Vogel, Mariana Trench. Only a representative portion of augite analyses are shown. Field (enclosed) for basaltic komatiites from ref. 27 and this work. Analyses by energy-dispersive electron probe operated at 20 kV and 30 nA. Trace elements mentioned in the text were determined on a Geoscan instrument operated at 20 kV.

Groundmass calcic pyroxenes, however, are ubiquitous and typically have skeletal forms²⁸ and metastable compositions, evidence of supercooling and rapid growth. In boninites, the bulk of the quench pyroxene is aluminous augite but in a few examples from each locality except Othris, pyroxene is zoned continuously from pigeonite to augite; spot analyses selected to show the range of compositions are given in Fig. 2.

Table 2 and Fig. 2 show that samples from the Western Pacific have a different compositional trend with crystal growth from those associated with ophiolites. The Bonin Islands calcic pyroxene trend is the most magnesium-rich of the Pacific types whereas the Cape Vogel one is the least. More magnesian by far is the Cyprus trend which meets the least calcic of the Othris pyroxenes at about Wo₃₈. The crystallisation trends are specific to each locality and probably represent differing thermal histories just before eruption as much as fundamental differences in parent liquid compositions.

Orthopyroxene is rare in komatiites and so far has been positively identified only as a cumulus phase in the thick submarine flows of basaltic komatiite composition from the Abitibi belt of Ontario^{24,27}. The variation in composition of groundmass pigeonite-augite is very similar to that of boninites. The data given in Fig. 2 are for pyroxenes in a pyroxene-spinifex top to a thin flow from the Belingwe belt and in ref. 24. Most basaltic komatiites, however, and all peridotitic komatiites, contain only augite and sub-calcic augite.

Deep red-brown magnesiochromites occur in almost every section of boninite or basaltic komatiite. Typically they are <0.03 mm across, euhedral and surrounded by glass. We detected zoning in one phenocryst only, from Mg/(Mg + Fe²⁺) = 0.683 at the centre to 0.669 on the margin. All spinels are very chromium rich (Fig. 3) and have Fe³⁺/(Fe³⁺ + Al + Cr) ratios <0.1. Those in boninites from the Western Pacific have higher Cr/(Cr + Al) ratios than those in the komatiite samples, whereas those from ophiolites span the whole range. Chromites in basaltic komatiites were found to have Mg/(Mg + Fe²⁺) < 0.6. Figure 3 emphasises the peculiar composition of these spinels contrasted with those found in typical Mid-Atlantic Ridge basalts²⁹⁻³¹; comparable compositions occur in the Great Dyke³² and the Troodos plutonic complex itself³³.

Basaltic komatiites of comparable MgO contents to boninites commonly have a groundmass of microlitic plagioclase (0.2 by <0.01 mm) which we have determined, in one sample, to be An₆₄. Samples from the cores of boninitic pillow lavas from Othris and Cyprus display a similar plagioclase morphology but only rarely is the feldspar unaltered. In the Upper Pillow Lavas

from Cyprus values of An₆₅₋₇₅ were obtained and more calcic compositions (up to An₉₅) have been reported from ultramafic lavas²⁵. Several boninite specimens from each locality, except the Bonin Islands, contained tiny laths, stellate aggregates or dendrites of green hornblende concentrated in the centres of pools of glass. The textures indicate that they are of primary origin, demonstrating the hydrous nature of the magma. In addition, rosettes of biotite up to 0.3 mm across occur in one specimen from the Mariana Trench¹⁵ and several of the ultramafic (olivine-phyric) lavas from Othris. Perfectly isotropic glass in the boninites has a water content of 6–8% (microprobe, by difference) but it is unlikely that all of it is original.

Primary hydrous phases are very rare in komatiites. Arndt²³ has described cumulus brown hornblende from close to the centre of one of the basaltic komatiite layered flows mentioned earlier but we could find no evidence of original groundmass hornblende in the least altered Rhodesian samples.

Boninites and komatiites are very similar in both mineralogy and texture. With the exception of the spinifex olivine rocks from Gorgona Island³⁴, which we do not consider are boninites because neither are they quartz normative nor do they have large modal amounts of hydrous glass, boninites are unique in this respect amongst Phanerozoic rocks. The only notable mineralogical differences between basaltic komatiites and boninites lie in the relative paucity of orthopyroxene and hornblende and high chrome contents of olivine in the former.

Chemical composition

The chemical compositions of boninites and basaltic komatiites are similar (Tables 1 and 2) and both have Mg/(Mg + Fe) ratios appropriate for primitive liquids in equilibrium with mantle olivine³⁵. The lavas of the Arakapas fault belt have been called basaltic komatiites, largely on geochemical grounds¹², but this was rejected⁴ because of the significantly lower incompatible element contents, notably titanium, of those rocks and what we term boninites from Cape Vogel and the Mariana Trench. The TiO₂ contents of the Othris samples (Table 2), however, assuming that titanium is a relatively immobile element during alteration, are comparable with those of basaltic komatiites, as are their CaO/Al₂O₃ ratios calculated from the less altered ultramafic (olivine-phyric) lavas. The CaO/Al₂O₃ variation between different boninites is faithfully recorded in the pyroxene compositions: in Othris there are no low-calcium pyroxenes or sub-calcic augites, in the Cape Vogel and Mariana Trench rocks clinopyroxene occurs while the Bonin and Cyprus

Table 2 Mineralogical and chemical characteristics of basaltic komatiites and boninites

	Northern Ontario	Belingwe	Cape Vogel	Mariana Trench	Bonin Islands	Cyprus	Othris
Olivine							
Phenocrysts	Fo ₈₉₋₈₇	—	—	Fo ₉₂	—	Fo ₉₃₋₉₁	Fo ₉₂₋₉₁
Microphenocrysts	—	Fo ₈₈	Fo ₉₂	Fo ₈₉₋₈₈	Fo ₈₉₋₈₇	Fo ₉₀₋₈₆	altd
Groundmass	altd	→Fo ₈₂	—	—	—	→Fo ₈₃	—
Calcium-poor pyroxene							
Phenocrysts	En ₈₈₋₈₂	—	En ₉₃₋₉₁	En ₉₁₋₈₈	En ₈₉₋₈₇	En ₈₉₋₈₈	—
Microphenocrysts	—	—	En ₉₂₋₈₆	En ₈₈₋₈₅	En ₈₈₋₈₅	En ₈₈₋₈₇	—
Groundmass	—	—	→En ₈₁	→En ₈₃	→En ₈₄	→En ₈₄	—
Pigeonite-Augite							
Groundmass	En ₇₈ Wo ₈ Fs ₁₄ – En ₄₀ Wo ₄₅ Fs ₁₅	En ₇₃ Wo ₁₂ Fs ₁₅ *– En ₄₀ Wo ₄₃ Fs ₁₇	En ₇₆ Wo ₄ Fs ₂₀ – En ₂₆ Wo ₄₄ Fs ₃₀	En ₈₀ Wo ₅ Fs ₁₅ En ₃₁ Wo ₄₄ Fs ₂₅	En ₈₁ Wo ₅ Fs ₁₄ – En ₄₂ Wo ₄₁ Fs ₁₇	En ₈₃ Wo ₅ Fs ₁₂ – En ₂₈ Wo ₅₀ Fs ₂₂	En ₅₄ Wo ₃₈ Fs ₈ – En ₃₂ Wo ₅₂ Fs ₁₆
Representative Compositions							
Mg/(Mg + Fe ²⁺)	0.70–0.74	0.67–0.75	0.73–0.83	0.72–0.79	0.71–0.77	0.65–0.77	altd
CaO/Al ₂ O ₃ (av.)	0.83	~0.80	0.55	0.53	0.53	0.83	>0.9
Ti (% av.)	~0.5	~0.60	0.33	0.19	0.24	0.28	0.7
Cr (p.p.m., av.)	1400?	~1000	850	820	710	780	970
Ni (p.p.m., av.)	640?	~400	300	280	250	340	330

altd, mineral in pseudomorph form only; —, mineral or pseudomorph not observed; *, larger crystal. Mineral compositions are from this study except for the Canadian data (refs 23, 24 and 27); whole rock compositions include data from refs 6, 9, 11, 12, 15, 21 and 23; Fe²⁺ calculated on the basis of Fe³⁺/(Fe²⁺ + Fe³⁺) = 0.1.

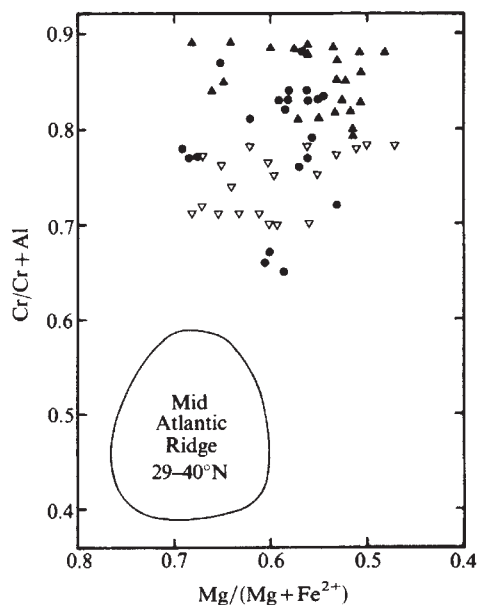


Fig. 3 Compositions of chromites from boninites (● and ▲ as Fig. 2) and basaltic komatiites (▽), projected onto the Fe^{3+} -free face of the spinel prism. Analyses by energy-dispersive electron probe, Fe^{2+} estimated by assuming stoichiometry. Field of Mid-Atlantic Ridge spinels from refs 29–31.

material has orthopyroxene as the low-calcium phase. In this context, basaltic komatiites are intermediate between the Cyprus and Othris examples. We conclude that a spectrum of titanium contents and $\text{CaO}/\text{Al}_2\text{O}_3$ ratios exists in boninites and each overlaps the range found in basaltic komatiites. This may result from differing degrees of melting, mantle inhomogeneity or chemical evolution of the mantle with time.

We are not entirely convinced that boninites have much lower Ti/Zr than chondritic values⁴. The Upper Pillow Lavas of the Troodos Massif, those in the Arakapas Fault belt and Agrilia Formation of the Othris Mountains have average Ti/Zr ratios of 100, 105 (ref. 12) and 94 respectively, close to chondritic values and basaltic komatiites (ranging from 60 to 140 in 50 samples).

Origin

Curiously, the origin of boninite would seem to be among the least controversial of any rock type. Green's carefully controlled experiments on water-saturated partial melting of pyrolite³⁶ produced a liquid at 1,200 °C, 10 kbar (~35% melting) which he calculated to have 54% SiO_2 , 14% MgO , an $\text{Mg}/(\text{Mg} + \text{Fe})$ ratio of 0.73 and $\text{CaO}/\text{Al}_2\text{O}_3 = 0.88$, the chief characteristics of boninite. Melting of a mantle segment that has been depleted in incompatible elements by previous melting episodes would require very high temperatures³⁷ to produce such magnesian liquids. It is difficult to imagine such temperatures in Phanerozoic mantle melts, thus we postulate that this could be

achieved only by a 'wet' process, whereas in the Archaean with less depleted mantle and possibly higher geothermal gradients, melts of similar or even more magnesian composition were generated by 'dry' melting. Basaltic komatiites were probably comagmatic with peridotitic komatiites, having arisen by low-pressure fractionation first of olivine, then augite from parental magmas with about 25% MgO ^{23,21,38}. Sun and Nesbitt suggested that the term komatiite should have the petrogenetic constraint that it is derived by dry melting of a mantle (with relatively undepleted incompatible element contents)⁴. Two observations are unexplained: the rare appearance of hornblende during fractional crystallisation of basaltic komatiites^{23,27} and the apparent scarcity of rocks with compositions lying between 16 and 20% MgO ; basaltic and peridotitic komatiite²¹.

There are great similarities between boninites and basaltic komatiites but the association of the latter with liquids of ultramafic composition is characteristic of the Archaean alone. It is tempting to speculate that the petrogenesis of basaltic komatiites and boninites differs from that of peridotitic komatiites by involving water, but the evidence presented above is inconclusive. We regard boninite therefore as petrographically, mineralogically and chemically the closest Phanerozoic equivalent to basaltic komatiite; the fundamental differences arising from changes in the Earth's mantle and thermal and tectonic regimes between the Archaean and the Phanerozoic.

It is significant that boninites are almost exclusively associated with ophiolites (but not necessarily vice versa). Troodos, Othris and the Canadian Palaeozoic examples^{17,19} form part of the extrusive sequence in well documented ophiolites. The igneous rocks from the Mariana dredge at 12° N are all of the 'ophiolite association'¹⁴.

Boninites from the Western Pacific are in identical fore-arc tectonic settings on the 'mid-slope high' (ref. 39). It is possible that boninites, and by implication ophiolites containing boninitic lavas, were formed *in situ* by partial melting of peridotite overlying the downgoing slab and that the associated two-pyroxene andesites and dacites⁵ are manifestations of a steadily evolving boninitic liquid from an island-arc setting⁶. There are two main problems with this view: the fact that the active volcanic chain in the Western Pacific is 150 km further away from the trench than the mid-slope high and that the overlying slab would be too cool. One possibility may be that boninites are erupted in the initial stages of breaking an older oceanic plate. It is very unlikely that boninites represent material scraped off the oceanic plate—they share none of the petrographic or geochemical characteristics of basalts generated at mid-ocean ridges over the past 180 Myr.

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- Kikuchi, Y. *J. coll. Sci. Imp. Univ., Jap.* **3**, 67–89 (1890).
- Petersen, J. *Jahrb. Hamb. Wiss. Anst.* **8**, 1–59; 341–349 (1891).
- Tröger, W. E. *Spezielle Petrographie der Eruptivgesteine* (Deutschen Mineralogischen Gesellschaft, Bonn, 1969).
- Sun, S.-S. & Nesbitt, R. W. *Geology* **6**, 689–693 (1978).
- Kuroda, N. & Shiraki, K. *Rep. Fac. Sci., Shizuoka Univ.* **10**, 145–155 (1975).
- Shiraki, K. & Kuroda, N. *J. Geogr. (Tokyo)* **86**, 34–50 (1977).
- Smith, J. E. & Davies, H. L. *Bur. Miner. Res. Aust. Bull.* **165**, 25–27 (1976).
- Dallwitz, W. B., Green, D. H. & Thompson, J. E. *J. Petrol.* **7**, 375–403 (1966).
- Dallwitz, W. B. *23rd Int. geol. Cong.* **2**, 229–242 (1968).
- Smewing, J. D., Simonian, K. O. & Gass, I. G. *Contr. Miner. Petrol.* **51**, 49–64 (1975).
- Smewing, J. D. & Potts, P. J. *Contr. Miner. Petrol.* **57**, 245–258 (1976).
- Simonian, K. O. & Gass, I. G. *Bull. Am. Geol. Soc.* **89**, 1220–1230 (1978).
- Smith, A. G. *et al. Eclogae geol. Helv.* **68**, 463–482 (1975).
- Anonymous. *Ophioliti* **2**, 137–168 (1977).
- Dietrich, V. J., Emmermann, R., Oberhänsli, R. & Puchelt, H. *Earth planet. Sci. Lett.* **39**, 127–144 (1978).
- Meijer, A. *Eos* **59**, 1182 (1978).
- Upadhyay, H. D. *Science* **202**, 1192–1195 (1978).

- Crawford, A. J. & Keays, R. R. *Earth planet. Sci. Lett.* **41**, 197–208 (1978).
- Gale, G. H. *Earth planet. Sci. Lett.* **18**, 22–28 (1973).
- Varne, R. & Brown, A. V. *Contr. Miner. Petrol.* **67**, 195–207 (1978).
- Nisbet, E. G., Bickle, M. J. & Martin, A. J. *Petrol.* **18**, 521–566 (1977).
- Bickle, M. J., Martin, A. & Nisbet, E. G. *Earth planet. Sci. Lett.* **27**, 155–162 (1975).
- Arndt, N. T., Naldrett, A. J. & Pyke, D. R. *J. Petrol.* **18**, 319–369 (1977).
- Arndt, N. T. *Carnegie Inst. Wash. Yb.* **76**, 494–502 (1977).
- Gass, I. G. *Geol. Mag.* **95**, 241–251 (1958).
- Searle, D. L. & Vokes, F. M. *Geol. Mag.* **106**, 515–530 (1969).
- Arndt, N. T. *Can. J. Earth Sci.* **14**, 2620–2637 (1977).
- Fleet, M. E. *Can. Mineral.* **13**, 336–341 (1975).
- Sigurdsson, H. & Schilling, J.-G. *Earth planet. Sci. Lett.* **29**, 7–20 (1976).
- Sigurdsson, H. *Init. Rep. DSDP* **37**, 883–891 (1977).
- Donaldson, C. H. & Brown, R. W. *Earth planet. Sci. Lett.* **37**, 81–89 (1977).
- Irvine, T. N. *Can. J. Earth Sci.* **4**, 71–103 (1967).
- Greenbaum, D. *Econ. Geol.* **72**, 1175–1194 (1977).
- Gansser, A., Dietrich, V. J. & Cameron, W. E. *Nature* **278**, 545–546 (1979).
- Roeder, P. L. & Emslie, R. F. *Contr. Miner. Petrol.* **29**, 275–289 (1970).
- Green, D. H. *Can. Miner.* **14**, 255–268 (1976).
- Green, D. H. *Geology* **3**, 15–18 (1975).
- Bickle, M. J., Ford, C. E. & Nisbet, E. G. *Earth planet. Sci. Lett.* **37**, 97–106 (1977).
- Karig, D. E. *Bull. Am. geol. Soc.* **82**, 323–344 (1971).

Disappearance of pink-pigmented *Globigerinoides ruber* at 120,000 yr BP in the Indian and Pacific Oceans

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The planktonic foraminiferal species Globigerinoides ruber with pink-pigmented tests occupied a worldwide warm-water belt during much of the Pleistocene. This variety was exterminated from the Indian and Pacific Oceans at about 120,000 yr BP, based on the oxygen isotope stratigraphy in 11 Indo-Pacific deep-sea cores, but it continued to live on to the present in the North and South Atlantic Ocean and the Mediterranean Sea. The disparate Atlantic and Indo-Pacific records reflect faunal provincialism that has been developing in the two regions since the Pliocene.

THE pink-pigmented variety of *Globigerinoides ruber* (d'Orbigny) forms a large proportion of the planktonic foraminiferal fauna of the modern Atlantic Ocean¹⁻¹¹ and Mediterranean Sea¹², but is completely absent from the present-day Indian and Pacific Oceans¹³⁻¹⁸. This foraminiferal variety did, however, occur in a circum-global warm-water belt during the late Pliocene and Pleistocene. Here we document the timing and geographic extent of the disappearance of pink-pigmented *G. ruber* as a synchronous extermination throughout the tropical and subtropical Indo-Pacific at 120,000 yr BP (the beginning of the last full interglacial and the lower part of oxygen isotope stage 5). We have relied on $\delta^{18}\text{O}$ curves for direct worldwide

correlation of this biostratigraphic event because the fluctuations of these curves reflect global ice volume¹⁹ and are nearly isochronous within the time constraints of ocean mixing²⁰. The apparent diachroneity of the Atlantic and Indo-Pacific stratigraphic records of coloured *G. ruber* since 120,000 yr BP may be related to faunal provincialism which has been developing in tropical latitudes since the Pliocene²¹.

Indo-Pacific sediments

Although pink-pigmented *G. ruber* has not been reported from either the plankton or Recent sediments of the Indian and Pacific Oceans, it is occasionally listed from the Pleistocene and rarely from older sediments. Cushman *et al.*²² and later Todd²³ reported small red and faintly pink specimens from Bikini and Eniwetok Atolls. Brönnimann and Resig²⁴ found pink *G. ruber* in the Pleistocene of Deep Sea Drilling Project Site 62 in the western equatorial Pacific and Jenkins and Orr²⁵ found it in the Pliocene and Pleistocene of eastern equatorial Pacific DSDP sites. Thompson²⁶ reported an acme of pink-pigmented *G. ruber* between solution cycles III_p and II_p (250,000–124,000 yr BP) in the western tropical Pacific, and placed the top of its range near the 6/5e oxygen isotope boundary.

We have taken samples at 10 cm or closer intervals for $\delta^{18}\text{O}$ measurements and total faunal analyses from many piston cores (Table 1; Fig. 1) to delineate the extermination datum level. The samples for isotopic analyses were roasted under vacuum at

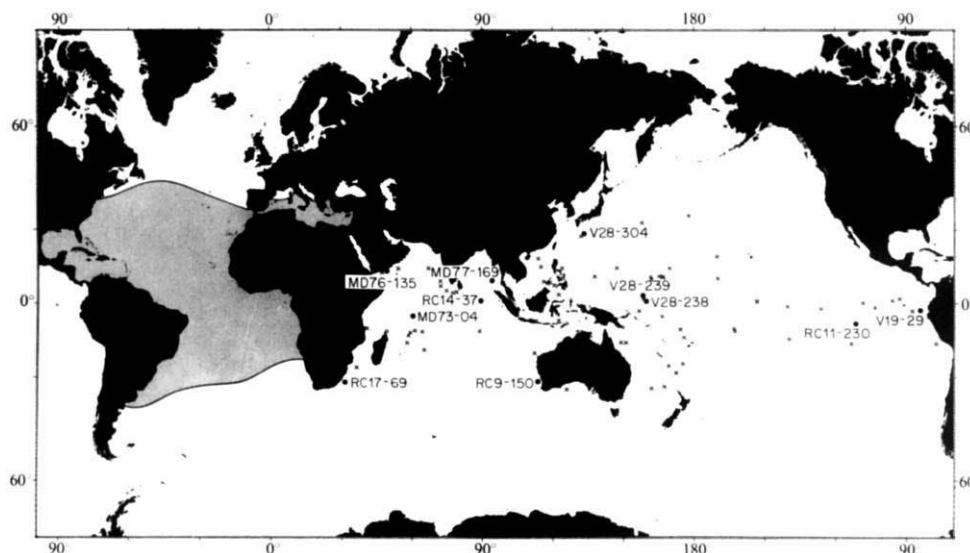


Fig. 1 Locations of piston cores with pink-pigmented *G. ruber* 120,000 yr BP datum and oxygen isotope records (dots), and other downcore occurrences of pink-pigmented *G. ruber* (crosses) in the Indo-Pacific. Pink-pigmented *G. ruber* survived in the Atlantic Ocean and Mediterranean Sea where it occurs in Quaternary sediments and present-day warm-water region (shaded).

Table 1 Cores used in this study

Core	Lat	Long	Water depth (m)	Core length (cm)	Isotopic reference	Occurrence level of 6/5e* boundary in core (cm)	Top occurrence of <i>G. ruber</i> † in core (cm)	Age of datum‡ (yr BP)
RC17-69	31°30' S	32°36' E	3,308	1,130	31	205	170–180	109,170 ± 3,120
MD76-135	14°27' N	50°31' E	1,895	1,125	Duplessy, this work	885	860–870	125,100 ± 720
MD73-04	04°58' S	61°40' E	3,440	1,700	Duplessy, this work	212	212	128,000
RC14-37	01°28' N	90°10' E	2,226	605	32	190	160–170	111,160 ± 3,370
MD77-169	10°12' N	95°03' E	2,360	1,460	Duplessy, this work	910	880–890	124,900 ± 1200
RC9-150	31°17' S	114°36' E	2,703	512	31	270	240–250	116,150 ± 2,370
V28-304	28°32' N	134°08' E	2,942	824	Shackleton, this work	455	426–430	120,460 ± 620
V28-238	01°01' N	160°20' E	3,120	1,609	19	217	201–202	119,760 ± 1,200
V28-239	03°15' N	159°11' E	3,490	2,102	30	127	110–120	115,900 ± 5,030
RC11-230	08°48' S	110°48' W	3,259	480	28	245	220–230	117,030 ± 2,090
V19-29	03°35' S	83°56' W	3,157	1,680	27	865	850–855	126,150 ± 370

* Given by Shackleton and Opdyke¹⁹ as 128,000 yr BP.

† Pink-pigmented form.

‡ Interpolated from 6/5e boundary and sample interval.

400 °C for 45 min and reacted under vacuum with 100% H₃PO₄ at 50 °C; the ¹⁸O/¹⁶O ratio is reported as δ¹⁸O relative to the Chicago Pee Dee Belemnite (PDB-1) standard:

$$\delta^{18}\text{O} = \left[\frac{(^{18}\text{O}/^{16}\text{O})_{\text{sample}}}{(^{18}\text{O}/^{16}\text{O})_{\text{standard}}} - 1 \right] \times 1,000$$

Faunal studies are based on a minimum count of 300 individuals greater than 149 µm in diameter to ensure statistical reproducibility. To take into account the effect of carbonate dissolution on the absolute abundances of total *G. ruber* populations through time²⁶, downcore variations have been expressed as the ratio of pink to total *G. ruber*.

The oxygen isotopic records^{27–32} and stratigraphic ranges of pink *G. ruber* for six Indian Ocean and five Pacific Ocean cores are shown in Figs 2 and 3. The curves were plotted to align isotopic stage 5e. Marked stage-to-stage sediment accumulation variations point to the effects of local depositional characteristics. The lithostratigraphic top of the range of pink *G. ruber* consistently lies within stage 5. Furthermore, cores with slow deposition rates, such as RC17-69, RC14-37, V28-238 and V28-239, show higher vertical occurrences than cores with high rates of accumulation such as MD76-135, MD77-169 and V19-29.

Isotopic measurements on both pink and white forms from stages 7 to 5e of core MD77-169 are shown in Fig. 4. Pink *G. ruber* is 0.17‰ lighter (s.d. 0.14) than the white form. Too few specimens were recovered from samples at the 5e maximum (880 and 890 cm) to analyse, but the light isotopic composition of the pink shells at 900 cm shows that these individuals lived after the 6/5e transition. The abundance of pink *G. ruber* shells decreases from 950 to 900 cm; at 900 cm, bioturbation will introduce isotopically heavy shells deposited in stage 6, but the same bioturbation cannot introduce isotopically lighter shells from higher up in stage 5e because the variety has already disappeared. Therefore, bioturbation will produce isotopically heavier measurements if the pink form is analysed rather than the white form, which can still be contaminated from stage 5. The true (chronostratigraphic) level for the top of the range of pink *G. ruber* is probably above the 5e/6 transition but before the 5d interglacial stadial. The occurrence of pink specimens above this level is attributed to sediment mixing by bioturbation^{33–35}. Thus, in cores with rapid sedimentation rates, the level of highest lithostratigraphic occurrence is closer to the true disappearance level than it is in cores with slower accumulation

rates, where an equivalent amount of bioturbation can disturb sediments representing a longer period of time.

The ratio of coloured-to-total *G. ruber* shows a consistent pattern down the cores. In the late portion of oxygen isotopic stage 6, the ratio averages 10%. It drops to 0% in the early part of stage 6, quickly returns to 10% or more in stage 7, and climbs to 20–25% in stage 8. In the two subtropical cores RC17-69 and RC9-150, the overall proportions of *G. ruber* are lower than in the more tropical cores reflecting the higher latitudes of the sites, but the downcore pattern of pink *G. ruber* ratios is similar to other cores studied. In all cores, abundances decrease rapidly below isotopic stage 10. In the early Brunhes and late Matuyama sections of V28-238 and V28-239 pink *G. ruber* was noted only from intervals of low carbonate dissolution. Specimens from these samples show a very faint colouration, fading downcore to the extent that they become indistinguishable from the colourless (white) form. Thus, we conclude that first occurrences in the middle of the Pleistocene do not necessarily mark an abrupt evolutionary appearance at this time, but are probably due to a progressive improvement in preservation of the pigment.

There is a possibility that pigmented and non-pigmented *G. ruber* may be differentiated on a morphological basis. If, as Hecht³⁶ suggests from a study of living Atlantic forms, the pigmented varieties are larger in size with wider apertural openings and a higher spire than the white (non-pigmented) type, this morphology may enable us eventually to place a lower age limit on the range of the coloured type despite its fading.

Atlantic sediments

In the North and South Atlantic, pigmented *G. ruber* occurs in the modern-day plankton and in sediments from middle Pleistocene to the Recent. It is most abundant in the boundary currents including the Gulf Stream, Antilles, Canaries, and Brazil Currents, and near Caribbean islands; it seems to be less abundant in regions away from land masses, such as the central Sargasso Sea, the central equatorial Atlantic, and the central South Atlantic^{1–11,37}. The distribution pattern generally coincides with temperature maxima of surficial waters, and seems to avoid upwelling areas^{38,39}. A strong seasonality has been observed in middle latitude regions^{40,41} where the white form predominates during the colder times of the year and the pink form in warmer periods. The pigment intensity increases from pink to deep ruby red with increasing temperatures. Bé and Tolderlund¹⁰ observed a seasonal 'migratory' trend of pigmented

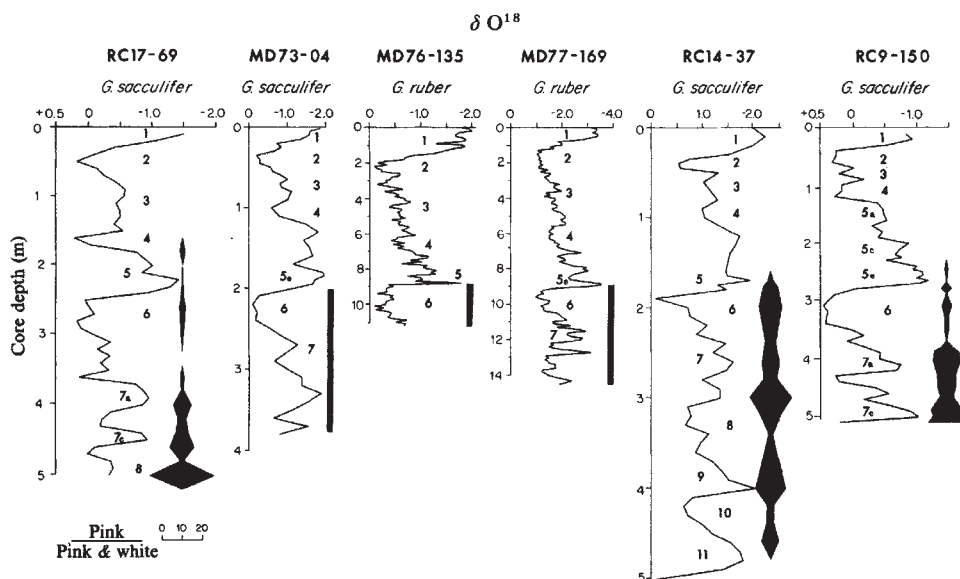


Fig. 2 Indian Ocean cores showing extinction datum of pink-pigmented *G. ruber* and oxygen isotope records (relative to PDB). Dark shading is the % ratio of pink to total *G. ruber*; light shaded line is occurrence of pink *G. ruber* in the core without quantitative measures.

G. ruber populations in the North Atlantic between the Caribbean and regions north of Bermuda. A front of pink *G. ruber* 'moved' northwards from spring to late autumn due to increasing numbers of pink *G. ruber* that developed pigmentation in response to locally elevated temperatures. A reverse southward 'migration' was observed from late autumn to early spring.

Surface sediment distributions of pigmented *G. ruber* closely follow plankton distributions in the North¹⁻¹¹ and South Atlantic (Fig. 1). The northern limit runs approximately from Nova Scotia in a slightly northerly bulging arc to Spain, whereas the southern limit runs obliquely from Golfo San Matias (Argentina) to Walvis Bay (Namibia). Pink *G. ruber* is absent south of Walvis Bay both in the plankton and surface sediments, indicating that a barrier presently exists which prevents the South Atlantic population from entering the Indian Ocean. Similarly, the South American continent's extension to 55°S latitude effectively blocks pink *G. ruber* from the Pacific Ocean. The highest proportions of the coloured variety underlie tropical areas of high surface temperature. Both pigmented and non-pigmented *G. ruber* occur in highest percentages on topo-

graphically shallow areas where carbonate dissolution is relatively low.

The tendency for pigmented tests of *G. ruber* to be associated with warm oceanic regions seems to be substantiated by downcore studies. Most studies that have distinguished pink from white *G. ruber* have noted two trends reflecting latitudinal occurrence. Low-latitude cores from the Gulf of Mexico, Caribbean and central Atlantic contain both types of *G. ruber* throughout glacial and interglacial stages⁴²⁻⁴⁵. Cores from subtropical and temperate regions, the Mediterranean⁴⁶, and the late Pliocene-early Pleistocene Le Castella section in Italy^{47,48}, contain pigmented forms only during warm, interglacial stages.

Discussion

The pigmentation in *G. ruber* has been preliminarily identified as the organic compound pheophytin, a carotene⁷, incorporated into the test wall. The intensity of colouration is greatest in the earliest-formed chambers and typically becomes progressively lighter in later chambers. The final chamber may vary from

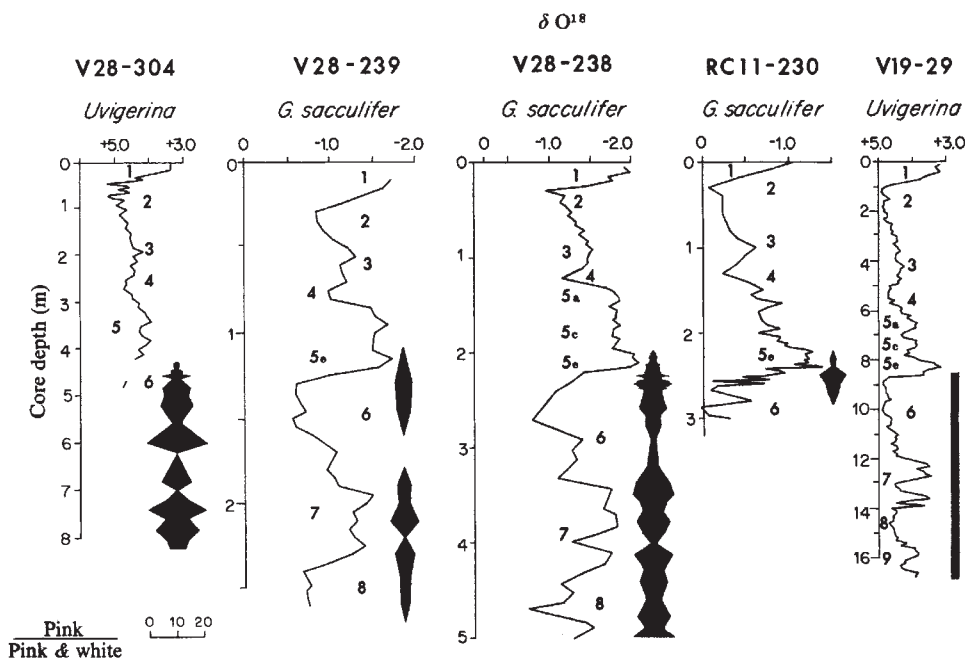


Fig. 3 Pacific Ocean cores with pink-pigmented *G. ruber* datum and oxygen isotopes. See also Fig. 2. Note that *Uvigerina* is absent during stage 5e (420-460 cm) in V28-304.

intensely pink to colourless. The origin and function of the pigmentation are unknown, but may be related to the large quantity of symbiotic zooxanthellae harboured within the protoplasm^{49,50}. This light-dependent symbiosis requires *G. ruber* to have a near-surface warm-water habitat, which has been verified by plankton tows^{7,10} and, ultimately, gives the species its relatively light oxygen isotopic composition^{51,52}.

In the Indo-Pacific sediments examined, the pigment intensity is generally much less than in the Atlantic. The Pacific forms are either light pink throughout or are light red in the early chambers, later becoming white. The deep red colouration and large test size common to the tropical Atlantic and West Indies are rare in the Pacific. We suggest that the intensity of colouration is related to vital (and perhaps genetic) factors controlling the production of the pheophytin such as nutrient supply, light intensity, surface temperature, and possibly symbiont type and proximity to land masses. Orr^{25,49} has also interpreted the intensity of pigmentation as a qualitative indicator of sedimentation rate.

The oxygen isotopic change of 1.5–1.8‰ in most records across the 6/5e transition demonstrates a drastic, worldwide ice-volume shift in a period of only several thousand years. Isotopic values (see Figs 2 and 3) indicate that stage 6 was one of the longest glacial periods of the Quaternary. The white *G. ruber* form can be found throughout the Pleistocene and Recent Indo-Pacific warm-water region and seems to have suffered no detrimental effects. The persistence of pink forms to the base of stage 5e (Fig. 4) indicates that it lasted through stage 6 to the time of initiation of stage 5 conditions. Again we return to the nature of the pigment and its source. If dinoflagellate symbionts produce the pigment, the extermination may be due to the elimination of the symbionts and thus their host, whereas the species of symbiont in white *G. ruber* would have been unaffected. If production of the pigment is operated by *G. ruber* itself, a case can be made that pink *G. ruber* may be a separate subspecies from the white form. In either case, the disappearance of pigmented *G. ruber* is an important palaeoecological indicator of some natural condition(s) that dropped below, or was raised above, a critical tolerance.

The absence of pink *G. ruber* in the Quaternary and modern Indo-Pacific region may be further evidence of a faunal provincialism that developed between the Atlantic and the Indo-Pacific Oceans after the separation of the Atlantic from the Pacific by the emergence of the Panama Isthmus in the late Pliocene²¹. Rare occurrences of pink *G. ruber* are reported from the late Pliocene from both sides of the Isthmus^{25,42,47}. Other evidence of Quaternary faunal provincialism is in a reversed sense of that of pink *G. ruber*. Thus, the planktonic foraminiferal species *G. adamsi*, *G. hexagona*, *G. conglomerata*^{45,53}, *G. pseudofoliata* (ref. 26) and *Globorotalia menardii neoflexuosa*^{54–56} occur in Quaternary sediments and present-day waters

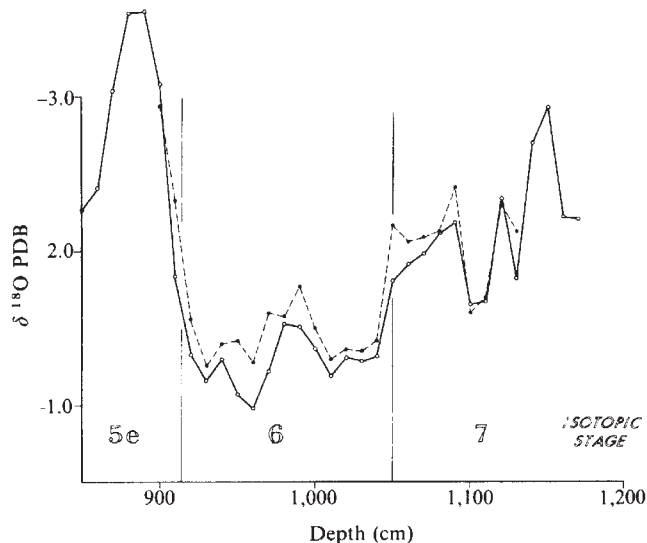


Fig. 4 Isotopic values of pink (●) and white (○) *G. ruber* in core MD77-169. The values show that the pink form lasted into stage 5e.

of the Indo-Pacific, but are absent in the Atlantic during this period. In addition, the coiling direction histories of *Pulleniatina* have diverged into two distinct patterns since the closing of the Panama Isthmus^{57,58}. We suspect that the Panama Isthmus emergence, the migrational barrier presented by the Agulhas Current flowing around the Cape of Good Hope with the relatively high latitudinal location of the southern boundary of the African continent at ~35°S, and the extension of South America to 55°S, may all have led to the development of genetic differences between Atlantic and Indo-Pacific populations of *G. ruber* which proved fatal to the latter during the earliest portion of oxygen isotope stage 5e. Their disappearance occurred soon after a rapid climatic transition which probably placed a certain stress on the population. Whatever the exact cause of the extermination of pink-pigmented *G. ruber* at 120,000 yr BP, stratigraphers now possess a most useful and widespread datum for the identification of the oxygen isotope 5e/6 transition throughout the Indo-Pacific area.

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- Brady, H. B. *Rep. Voy. Challenger, Zool.* **9**, 1–814 (1884).
- Cushman, J. A. *U.S. nat. Mus., Bull.* **104**, 1–45 (1924).
- Schott, W. *Deutsch. Atlant. Exped. Meteor.* 1925–1927. *Wiss. Ergebn.* **3**, 43–134 (1935); *Swedish Deep-Sea Exped. Rep.* **7**(8–7), 357–469 (1966).
- Phleger, F. B., Parker, F. L. & Pierson, J. F. *Swedish Deep-Sea Exped. Rep.* **7**(1), 1–22 (1953).
- Cifelli, R. *Smithsonian Inst., Misc. Coll.* **148**, 1–36 (1965).
- Bolotovskoy, E. *Argentina, Serv. Hidrogr. Naval, Publ. H* 640, 1–69 (1966); *Rev. Micropal.* **11**, 85–98 (1968).
- Bé, A. W. H. & Hamlin, W. H. *Micropaleontology* **13**, 87–106 (1967).
- Jones, J. I. *Micropaleontology* **13**, 489–501 (1969).
- Lynts, G. W. *Micropaleontology* **17**, 152–166 (1971).
- Bé, A. W. H. & Tolderlund, D. in *The Micropaleontology of Oceans* (eds Funnell, B. M. & Riedel, W. R.) 105–149 (Cambridge University Press, 1971).
- Kipp, N. V. *Mem. geol. Soc. Am.* **145**, 3–41 (1976).
- Thunell, R. C. *Marine Micropaleont.* **3**, 147–174 (1978).
- Bradshaw, J. S. *Cushman Fdn Forum. Res. Contr.* **10**, 25–64 (1959).
- Parker, F. L. *Micropaleontology* **8**, 219–254 (1962).
- Belyaeva, N. V. *Oceanology* **7**, 500–507 (1967).
- Berger, W. H. *Limn. Oceanogr.* **15**, 183–204 (1970).
- Savin, S. M. & Douglas, R. G. *Bull. geol. Soc. Am.* **84**, 2327–2342 (1973).
- Bé, A. W. H. & Hutson, W. H. *Micropaleontology* **23**, 369–414 (1977).
- Shackleton, N. J. & Opdyke, N. D. *Quat. Res.* **3**, 39–55 (1973).
- Broecker, W. S. *Chemical Oceanography* (Harcourt Brace Jovanovich, New York, 1974).
- Kennett, J. P. in *Foraminifera 2* (eds Hedley, R. H. & Adams, C. G.) 111–170 (Academic, New York, 1976).

- Cushman, J. A., Todd, R. & Post, R. J. *U. S. Geol. Surv., Prof. Paper* 260-H, 319–384 (1954).
- Todd, R. *U.S. Geol. Surv., Prof. Paper* 260-CC, 1067–1100 (1964).
- Brönnimann, P. & Resig, J. *Init. Rep. DSDP* **7**, 1235–1469 (1971).
- Jenkins, D. G. & Orr, W. N. *Init. Rep. DSDP* **9**, 1059–1193 (1972).
- Thompson, P. R. *J. Foramin. Res.* **6**, 208–227 (1976).
- Ninkovich, D. & Shackleton, N. J. *Earth planet. Sci. Lett.* **27**, 20–34 (1975).
- Luz, B. & Shackleton, N. J. *Cushman Fdn Forum. Res., Spec. Publ.* **13**, 142–150 (1975).
- Hays, J. D. & Shackleton, N. J. *Geology* **4**, 649–652 (1976).
- Shackleton, N. J. & Opdyke, N. D. *Mem. geol. Soc. Am.* **145**, 449–464 (1976).
- Bé, A. W. H. & Duplessy, J.-C. *Science* **194**, 419–422 (1976).
- Thierstein, H. R., Geitzenauer, K. R., Molino, B. & Shackleton, N. J. *Geology* **5**, 400–404 (1977).
- Berger, W. H. & Heath, F. R. *J. mar. Res.* **26**, 134–143 (1968).
- Duplessy, J.-C. in *Climatic Change* (ed. Gribbin, J.) 46–47 (Cambridge University Press, 1978).
- Thompson, P. R. & Sciarillo, J. R. *Nature* **276**, 29–33 (1978).
- Hecht, A. D. in *Foraminifera 2* (eds Hedley, R. H. & Adams, C. G.) 1–44 (Academic, New York, 1976).
- Adegoke, O. S., Dessauvage, T. F. J. & Kogbe, C. A. *Micropaleontology* **17**, 197–213 (1971).
- Thiede, J. *Nature* **253**, 712–714 (1975).
- Berger, W. H., Diester-Haass, L. & Killingley, J. S. *Oceanol. Acta* **1**, 3–7 (1978).
- Bé, A. W. H. *Micropaleontology* **6**, 373–392 (1960).
- Cifelli, R. *J. mar. Res.* **20**, 201–213 (1962).
- Bolli, H. M. *Init. Rep. DSDP* **4**, 557–643 (1970).
- Smith, L. A. & Beard, J. H. *Init. Rep. DSDP* **10**, 643–677 (1973).
- Rögl, F. & Bolli, H. M. *Init. Rep. DSDP* **15**, 553–615 (1973).
- Bé, A. W. H., Damuth, J. E., Lott, L. & Free, R. *Mem. geol. Soc. Am.* **145**, 165–200 (1976).

46. Vergnaud-Grazzini, C. & Herman-Rosenberg, Y. *Rev. Géogr. Phys. Géol. Dynam.* (2) **9**, 279–292 (1969).
47. Emiliani, C. *Micropaleontology* **15**, 1–22 (1969); *Micropaleontology* **17**, 233–237 (1971).
48. Diester-Haass, L., Schrader, H.-J. & Thiede, J. *Meteor. Forsch.-Ergebn.* **C16**, 19–66 (1973).
49. Orr, W. N. *Micropaleontology* **15**, 373–379 (1969).
50. Tolderlund, D. & Bé, A. W. H. *Micropaleontology* **17**, 297–329 (1971).
51. Emiliani, C. *Am. J. Sci.* **252**, 149–158 (1954).
52. Shackleton, N. J., Wiseman, J. D. H. & Buckley, H. A. *Nature* **242**, 177–179 (1973).
53. Parker, F. L. *Bull. Am. Paleont.* **52**, 115–208 (1967); in *The Micropaleontology of Oceans* (eds Funnell, B. M. & Riedel, W. R.) 289–307 (Cambridge University Press, 1971).
54. Bé, A. W. H. & McIntyre, A. *Deep-Sea Res.* **17**, 595–601 (1970).
55. Srinivasan, M. S., Kennett, J. P. & Bé, A. W. H. *Deep-Sea Res.* **21**, 321–324 (1974).
56. Adelseck, C. G. Jr *Deep-Sea Res.* **22**, 689–691 (1975).
57. Saito, T. *Geology* **4**, 305–309 (1976).
58. Keigwin, L. D. Jr *Geology* **6**, 630–634 (1978).

Temperature-dependent X-ray diffraction as a probe of protein structural dynamics

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X-ray diffraction at four temperatures from 220 to 300 K coupled with crystallographic refinement yields the mean-square displacements and conformational potentials of all 1,261 non-hydrogen atoms of metmyoglobin. The results are interpreted to indicate a condensed core around the haem, semi-liquid regions towards the outside and a possible pathway for ligands. It is concluded that X-ray diffraction can provide the spatial distribution of the dynamic features of a protein.

TRADITIONAL X-ray models present proteins as static molecules of unique structure. Experimental findings and theoretical arguments, however, indicate that they are fluctuating systems^{1,2}. A protein in an equilibrium conformation can exist in a very large number of conformational substates; proteins in different substates have the same coarse structure but differ in local configurations. Rotations about single bonds or shifts of hydrogen bonds may occur. The experiments of Austin *et al.* imply that all conformational substates have the same biological function, but possibly at different rates³. As transition from one substate to another generally occurs by overcoming a potential energy barrier, the dynamic behaviour of proteins depends on temperature. At sufficiently high temperatures, a protein molecule moves rapidly from one substate to another, breaking and reforming non-covalent bonds and changing the structure continuously. At low temperatures, much of this conformational relaxation is lost and each molecule remains in a particular substate. The experimental evidence for a fluctuating protein comes from many different techniques. Flash photolysis data at low temperatures imply the existence of many conformational substates^{3,4}. Intramolecular motions have been inferred from fluorescence quenching⁵ and relaxation⁶, NMR^{7–9}, and isotope exchange^{10,11}. Theory implies that a molecular system of N components (amino acids) has approximately e^N states with energies close to that of the ground state¹² and experiences sizeable fluctuation in internal energy, entropy and volume¹³. Because binding is 'strong' ($\gg k_B T$) along the polypeptide backbone and 'weak' ($\approx k_B T$) in other directions, a biopolymer may form a globule, with a condensed core and a diffuse outer phase¹⁴. A computer solution of the equations of motion of the atoms in a globular protein suggests a fluid-like interior, atoms displaced from their equilibrium position by up to 2 Å, and fluctuations on a picosecond time scale¹⁵.

The experimental evidence for the effect of substates on the binding of carbon monoxide to myoglobin³ has prompted us to investigate whether conformational substates can be observed

by X-ray diffraction. Consider a typical globular protein such as myoglobin. X-ray diffraction permits the determination of the mean-square displacement, $\langle x^2 \rangle$, of all atoms except hydrogen. The temperature dependence of $\langle x^2 \rangle$ should reveal regions of conformational flexibility. In this article, we outline the features underlying the determination of $\langle x^2 \rangle$, describe the experimental approach and present data which support the picture of a fluctuating protein.

Atomic displacements from X-ray scattering

X-ray scattering can determine not only the position, but also the mean-square displacement, $\langle x^2 \rangle$, of a scattering particle¹⁶. For an atom in a protein crystal, $\langle x^2 \rangle$ can at a first approximation be written as

$$\langle x^2 \rangle = \langle x^2 \rangle_c + \langle x^2 \rangle_d + \langle x^2 \rangle_{ld} + \langle x^2 \rangle_v \quad (1)$$

The subscripts stand for conformation substates, diffusion, lattice disorder and vibration, respectively. The term $\langle x^2 \rangle_d$ is due to translational and rotational diffusion and depends on T/η , where T is the temperature and η the viscosity of the crystal. The term $\langle x^2 \rangle_{ld}$ is caused by macroscopic disorder in the protein crystal. Even if all protein molecules were identical, the crystal would possess some disorder. Disorder should be almost temperature independent and, if purely translational, contribute equally to all atoms in the protein. The vibrational term, $\langle x^2 \rangle_v$, contains components from individual atoms¹⁷, groups of atoms, and the entire molecule (rigid-body motion). The main contribution comes from the vibration of groups of atoms. In the Debye approximation, $\langle x^2 \rangle_v$ is constant well below the Debye temperature T_D and proportional to T above T_D (Fig. 1b)¹⁶.

The conformational term, $\langle x^2 \rangle_c$, is of central interest here. If proteins are fluctuating systems with many conformational substates, each atom or group of atoms will be able to occupy different substates, as shown in Fig. 1a, which gives the conformational potential, V_c , as a function of the distance from the mean position. The substates correspond to local minima in V_c . Above a relaxation temperature, T_r , each atom will rapidly move from one substate to another; the substates are in equilibrium and populated according to a Boltzmann distribution. Below T_r the atoms can no longer overcome the barriers between substates and each atom remains in a particular substate. X rays interact with a rapidly fluctuating and a 'frozen' protein in the same way: X-ray diffraction measures the spatial distribution over conformational substates in the fluctuating and the frozen state. The temperature dependence expected for $\langle x^2 \rangle_v$, for $\langle x^2 \rangle_c$, and for their sum is shown in Fig. 1b. The conformational potentials and the barriers between substates depend on the local structure and may change with temperature. The relaxation temperature may differ from atom to atom; the value indicated in Fig. 1b implies an average.

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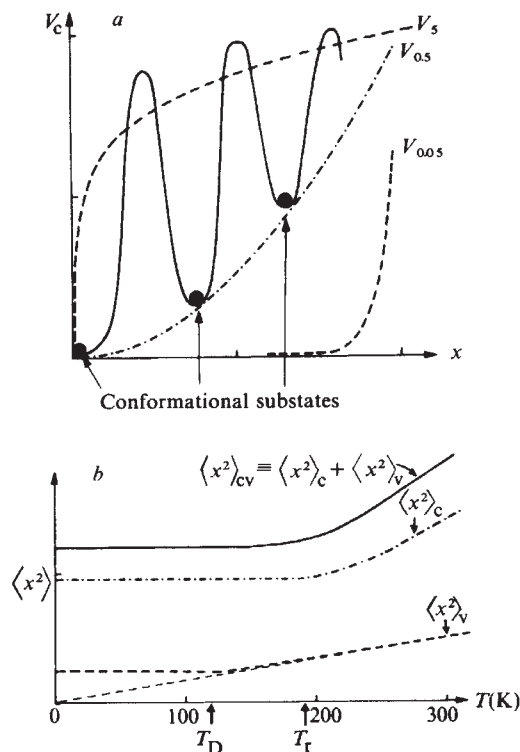


Fig. 1 *a*, The local minima in the conformational potential V_c , given by the solid line, represent conformational substates. The bottoms of the wells are parametrised by power laws, $V_c \propto x^{1/\nu}$, and three cases are shown as dashed lines. *b*, Temperature dependence of the conformational ($\langle x^2 \rangle_c$) and vibrational ($\langle x^2 \rangle_v$) mean-square displacement.

The distinction between vibrational and conformational contributions to $\langle x^2 \rangle$ is to a certain extent a matter of definition. One way of separating the two terms is by their low-temperature limit. Vibrational contributions should converge towards a value typical for zero-point vibrations, whereas conformational substates can lead to a much larger value of $\langle x^2 \rangle$.

Mössbauer effect

Independent information on $\langle x^2 \rangle$ for some nuclei (for example, ^{57}Fe) comes from the recoil-less fraction, f , of the Mössbauer effect^{18,19}. Each nucleus absorbs randomly, stationary disorder does not decrease f , and only conformational substates, diffusion²⁰ and vibrations must be considered. The time scale in the Mössbauer effect is set by the nuclear lifetime, τ_N (140 ns for ^{57}Fe); $\tau_c(T)$ will denote the temperature-dependent mean time for conformational relaxation. At a characteristic temperature T_c , τ_c is equal to the nuclear lifetime

$$\tau_c(T_c) = \tau_N \quad (2)$$

Well below T_c , τ_c is very long relative to τ_N and the Mössbauer effect is sensitive only to $\langle x^2 \rangle_v$.

$$\langle x^2 \rangle_{\text{Mö}}(T \ll T_c) = \langle x^2 \rangle_v \quad (3)$$

Well above T_c , τ_c is short relative to τ_N and the Mössbauer effect is sensitive to all terms in equation (1) except $\langle x^2 \rangle_{\text{id}}$.

$$\langle x^2 \rangle_{\text{Mö}}(T \gg T_c) = \langle x^2 \rangle_c + \langle x^2 \rangle_d + \langle x^2 \rangle_v \quad (4)$$

Data collection and structure refinement

Crystals of metmyoglobin have the symmetry of space group $P2_1$, with cell dimensions $a = 64.31 \text{ \AA}$, $b = 30.85 \text{ \AA}$, $c = 34.85 \text{ \AA}$, $\beta = 105.85^\circ$, and one molecule of molecular weight 17,900 per asymmetric unit. One crystal of dimensions $1.1 \times 0.8 \times 0.5 \text{ mm}^3$ was used for the measurements at 300 K, 275 K

and 250 K. The crystal was in contact with its normal mother liquor containing 75% saturated ammonium sulphate and 0.1 M phosphate, pH 6.1. Measurements at 200 K were made on a different crystal of identical size and shape in which the normal mother liquor was replaced by 75% 2-methyl-2,4-pentanediol, 30% water and 0.02 M cacodylate buffer with proton activity $\text{pH}^* = 6.1$ (ref. 21). This mixed solvent is a cryoprotectant for the crystals and permits measurements below the freezing point of the ammonium sulphate mother liquor²².

X-ray diffraction data were recorded with a Syntex P2₁ diffractometer equipped with an LT-1 low-temperature device, both modified for protein crystallography. Data were collected by a limited ω -step-scan procedure with continuous checking for crystal movement. No movement was detected. An empirical background curve was fitted to the data²³, which were also corrected for absorption²⁴. The crystals were chosen so that the absorption effect was small. The data set at each temperature consisted of over 22,000 independent measurements to 1.5 Å resolution. Only reflections with intensities greater than twice their estimated standard deviations were retained for the refinements, approximately 16,000 per data set. To ensure that the effects of radiation damage were not misinterpreted as increased B factors on going to 300 K, data at 300 K were collected first, followed by the sets at 250 K and 275 K. Only 4% radiation-induced decay in diffraction intensities was observed at 300 K, and no significant radiation damage was detected at the lower temperatures.

Refinement of the metmyoglobin structure by the method of Konner²⁵ as programmed by Hendrickson (personal communication) involves least-squares minimisation of the weighted sums of $|F_o - F_c|$ plus $|d_o - d_c|^2$. F denotes the structure factor, d the inter-atomic distance; the subscripts indicate observed and calculated. Proper choice of the relative weights produces a restrained least-squares fit in which the atoms are tethered to positions that maintain ideal bond lengths and angles. Refinement with restraints provides a sufficient ratio of observations to variable parameters to allow the refinement of positional coordinates and an isotropic displacement parameter, $B = 8\pi^2 \langle x^2 \rangle$, for every non-hydrogen atom. The starting point at each temperature was the refined 2.0 Å resolution structure of metmyoglobin determined by Takano²⁶, with a discrepancy index

$$R = \sum_{hkl} |F_o - F_c| / \sum_{hkl} F_o = 0.26$$

The structures have each been refined at 1.5 Å resolution to $R = 0.18$; the refinements took different numbers of cycles to reach this point, but were terminated at the same R to make the standard deviations comparable. Restraints on covalent and non-covalent bond distances have been set to preserve ideality within about 0.05 Å for covalent distances and 0.1 Å for the others. Side-chain atoms involved in restrained interactions will have $\langle x^2 \rangle$ values that are correlated and possibly too small. Main-chain correlations might lead to $\langle x^2 \rangle$ values systematically too large. Ordered water was observed on the surface of the protein, frequently in positions identical to those found by Takano²⁶. These atoms were included in the refinement, but were not constrained.

Occasionally, the displacement parameters for a few atoms were found to increase with decreasing temperature, suggesting that the average position of the atom had changed, and the position being used in the refinement was no longer correct. This

Table 1 Average side-chain displacements at 250 K

Residue type	$\langle x^2 \rangle_{cv} (\text{\AA}^2)$	
	Outside	Inside
Charged	0.20 ± 0.02	0.10 ± 0.02
Neutral polar	0.16 ± 0.04	0.08 ± 0.04
Nonpolar	0.07 ± 0.02	0.05 ± 0.01

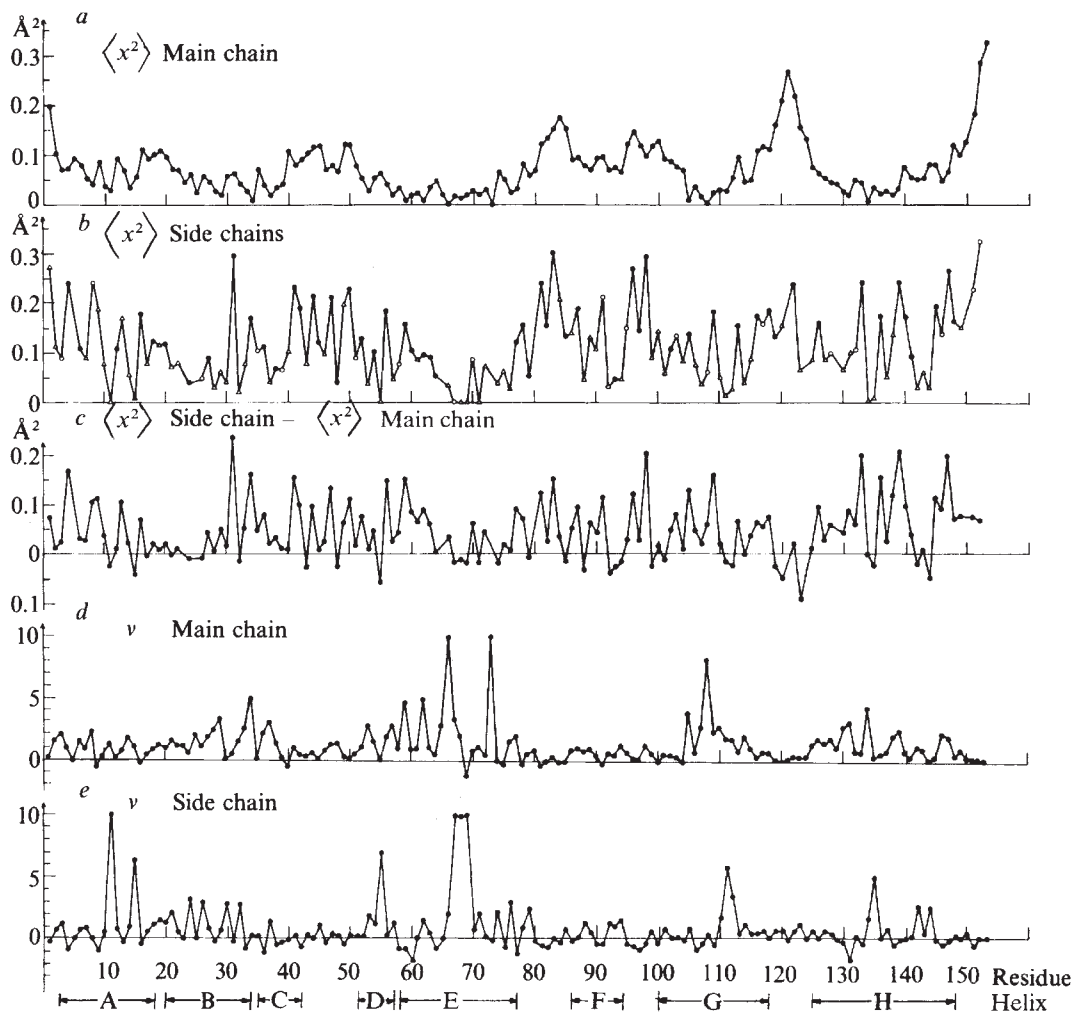


Fig. 2 *a*, The sum of the conformational and vibrational mean-square displacements, $\langle x^2 \rangle_{\text{cv}} = \langle x^2 \rangle_{\text{c}} + \langle x^2 \rangle_{\text{v}}$, averaged over the three main-chain atoms N, C α and C, is plotted against residue number for myoglobin. *b*, The largest displacement in each side chain is plotted against residue number. ●, Hydrophilic; ○, neutral; △, hydrophobic residues. *c*, Difference between side-chain and backbone displacement. *d*, Exponent ν_b in the fit $\langle x^2(T) \rangle_{\text{cv}} = \text{const. } T^{2\nu}$, plotted against residue number for the main chain (backbone). *e*, Exponent ν_s for the largest side-chain displacement.

suspicion was confirmed by inspection of difference electron-density maps, which were calculated at several stages in the refinement process. Manual correction of the atomic positions led to reasonable temperature dependence in the next cycles of refinement.

In some cases, particularly for the 220 K structure, we observed the appearance of a new electron density at low temperature in positions suggestive of alternative conformations for side-chain atoms. Variable occupancy terms in the refinement may deal with this situation. Difference maps calculated at the present stage of refinement are virtually featureless for the 300 K, 275 K and 250 K structures.

The reliability of the displacement parameters has been checked by refining certain atoms, such as the iron, in a conventional, unrestrained least-squares refinement program. Over-determination was achieved by fixing the remainder of the structure. Cycles of restrained refinement have also been carried out after removal of the restraints on displacement parameters. Finally, a second set of 250 K data from a different crystal was measured and used to obtain refined parameters for comparison. From these results we estimate the standard deviation of our mean-square displacements to be 0.015 Å² for most atoms.

Determination of $\langle x^2 \rangle_{\text{cv}}$

The evaluation described above gives $\langle x^2 \rangle$ for all non-hydrogen atoms. Two assumptions underlie the standard treatment and are built into the current refinement methods: (1) $\langle x^2 \rangle$ is isotropic; (2) the probability of finding a given atom a distance x from its mean position is gaussian

$$P_{1/2}(x) = P_{1/2}(0) \exp(-x^2/2\langle x^2 \rangle) \quad (5)$$

For the exploratory extraction of $\langle x^2 \rangle_{\text{c}}$, we add three more assumptions: (3) equation (1) holds; (4) diffusion can be neglected in crystals; $\langle x^2 \rangle_{\text{d}} = 0$; (5) all atoms have the same temperature-independent value of $\langle x^2 \rangle_{\text{ld}}$.

Assumptions (1) and (2) are certainly oversimplifications, but we retain them for the present. Assumption (4) is justified by the fact that the X-ray pattern is sharp even at 300 K. Assumption (5) is equivalent to considering the lattice disorder to be purely translational. Although we expect rotational disorder to be present, we have not yet assessed its magnitude. The fact that atoms on the protein surface have widely differing values of $\langle x^2 \rangle$ indicates, however, that rotational disorder cannot be dominant.

Our eventual goal is the separate determination of $\langle x^2 \rangle_{\text{c}}$ and $\langle x^2 \rangle_{\text{v}}$. In the present study we will only determine their sum, $\langle x^2 \rangle_{\text{c}} + \langle x^2 \rangle_{\text{v}} = \langle x^2 \rangle_{\text{cv}}$. To extract $\langle x^2 \rangle_{\text{cv}}$ from $\langle x^2 \rangle$, we combine the results from X-ray and Mössbauer experiments and use the haem iron as calibration. Equations (1) and (4) show that $\langle x^2 \rangle - \langle x^2 \rangle_{\text{M6}}(T \gg T_f) = \langle x^2 \rangle_{\text{ld}}$. Parak (ref. 27 and personal communication) has determined $\langle x^2 \rangle_{\text{M6}}$ in metmyoglobin crystals up to 283 K, obtaining values of 0.037 Å², 0.061 Å² and 0.068 Å² at 250 K, 275 K and 300 K, respectively. Our X-ray data give $\langle x^2 \rangle$ values of 0.091 ± 0.003 Å², 0.101 ± 0.003 Å² and 0.108 ± 0.003 Å², respectively, leading to $\langle x^2 \rangle_{\text{ld}} = 0.045 \pm 0.006$ Å². Subtraction of this value from the measured X-ray results yields $\langle x^2 \rangle_{\text{cv}}$ for all 1,261 non-hydrogen atoms of metmyoglobin. A table of all results at the four temperatures would be too long, and so we present in Fig. 2 only two numbers for each amino acid, the average for the backbone atoms N, C α and C, and the value for the atom with the largest displacement in each side chain at 250 K.

As an indication of the size of $\langle x^2 \rangle_v$, we note that linear extrapolation of the low-temperature Mössbauer data of Debrunner and coworkers²⁸ yields $\langle x^2 \rangle_v \approx 0.02 \text{ Å}^2$ at 300 K for the iron atom.

Structural dynamics of myoglobin

The data in Fig. 2 show that X-ray diffraction can provide an insight into the spatial distribution of the dynamic aspects of biomolecules. Phillips and coworkers have drawn similar conclusions^{29,30}.

Figure 2 provides evidence for conformational substates because $\langle x^2 \rangle_{cv}$ reaches large values relative to typical vibrational displacements, and the large displacements are essentially temperature independent. Our Fig. 2 shows features similar to the prediction displayed in Fig. 2 of the paper by Karplus and coworkers¹⁵. Both prediction and experiment show very large displacements at the two terminals, the largest one occurring at the carboxyl end. To find a lower limit for the number of substates, in Fig. 2c the difference Δ between the values of $\langle x^2 \rangle_{cv}$ for the backbone and the side chain are plotted for each residue as given in Fig. 2a and b. We arbitrarily assume that any residue with $\Delta \geq 0.025 \text{ Å}^2$ has at least two conformational substates. About 95 residues satisfy this criterion, yielding $\geq 2^{95} \approx 10^{28}$ substates.

Figure 3 shows the static myoglobin structure as solid lines, areas reached by movements (at 250 K) of the backbone atoms shaded. The limiting excursion x_{lim} region is determined by setting $P_{1/2}(x_{lim})/P_{1/2}(0) = 0.01$ (equation (5)). Conformational substates still have a probability of 0.01 of being outside the shaded volume.

Figures 2 and 3 provide information on the conformational substructure of myoglobin. Figure 2b indicates that 44 residues have a maximum side-chain mean-square displacement larger than 0.15 Å^2 . Almost all these lie on the surface of the protein. Of the 44, only 7 are hydrophobic. The observation of large values for $\langle x^2 \rangle_{cv}$ on the surface implies a considerable interaction between solvent and protein. Table 1 gives the average values for the largest side-chain displacements, grouped according to type and position of the residue. Not surprisingly, displacements

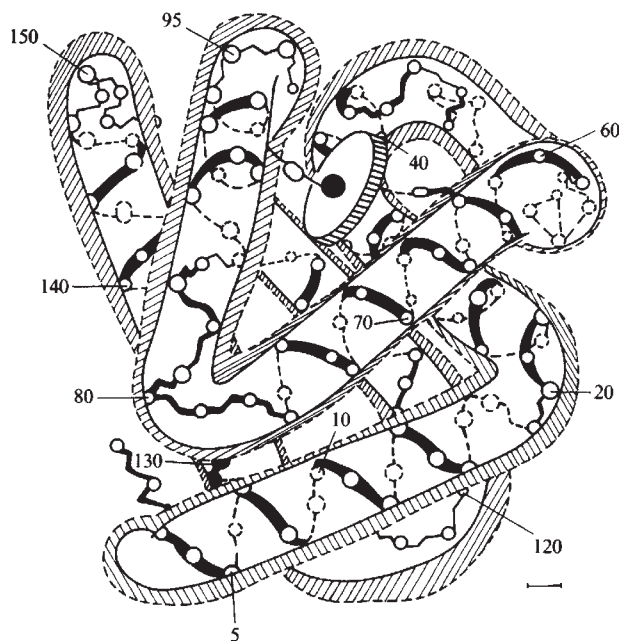


Fig. 3 Backbone (main chain) structure of myoglobin. The solid lines indicate the static structure as given in ref. 37. Circles denote the C α carbons; some residue numbers are given. The shaded area gives the region reached by conformational substates with a 99% probability. Scale bar, 2 Å.

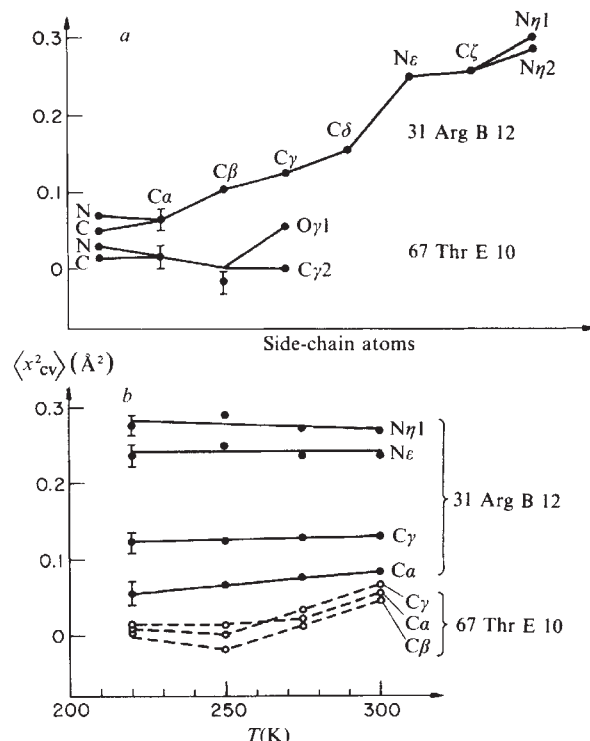


Fig. 4 Displacements $\langle x^2 \rangle_{cv}$ for all non-hydrogen atoms of Arg 31 and Thr 67. *a* Shows the displacement at 250 K as a function of distance from the main chain; *b* gives the temperature dependence for some of the atoms. All points have the same estimated error; error bars are only shown on some.

are smaller inside the protein than outside. Charged and polar residues show larger displacements than the nonpolar ones, possibly because of charge fluctuations in the solvent and the hydration shell^{1,2}.

It is useful to follow the main chain, comparing $\langle x^2 \rangle$ for both backbone and side chain with the position along the main chain. As already pointed out, the displacement is large whenever the side chain can extend into the solvent and small when other parts of the protein hinder the motion, as, for example, near residues 30, 60, 105 or 134. Even turns around the helix can be followed. Good examples are residues 10–14 in the A-helix, 25–28 in the B-helix and 73–76 in the H-helix, where the variation of $\langle x^2 \rangle_{cv}$ around the helix is clearly seen. Similar periodicities occur in many places, suggesting that the helices do not move only as rigid units, but may also experience rippling or breathing modes. The accompanying article presents similar results for lysozyme³⁰.

The region around the haem is particularly interesting. On the distal side, near His 64, residues 39, 64, 67, 68, 71, 72 and 107 are in van der Waals contact with the haem group. The average $\langle x^2 \rangle_{cv}$ at 300 K for the side chains is 0.04 Å^2 , so that $x_{r.m.s.} = 0.2 \text{ Å}$, comparable to values in many solids. These residues thus form a tight compartment around the binding site, guarding the access to the haem iron. Entrance to the pocket may be possible near residues 43–45, which are also on the distal side and have an average $\langle x^2 \rangle_{cv} = 0.15 \text{ Å}^2$. These residues are in the general region of one of the pathways found in a model calculation by Karplus and collaborators (D. A. Case and M. Karplus, in preparation). On the proximal side, near His 93, the situation is strikingly different. The average $\langle x^2 \rangle_{cv}$ for the side chains in contact with the haem (42, 43, 89, 92, 97, 99, 103, 104, 138) is 0.12 Å^2 . Moreover, the motion of the F-helix is also large. For haemoglobin, Perutz has suggested that this region is involved in a dynamic control mechanism³¹. The conformational displacement of the haem iron can be compared with the distribution in tunnelling distance observed in low-temperature flash photolysis³². If the conformational motion of Fe is perpendicular to the haem plane, $\langle x^2 \rangle_{cv}(\text{Fe}) = 0.04 \text{ Å}^2$ leads to $x_{r.m.s.}(\text{Fe}) = 0.35 \text{ Å}$.

The tunnelling data imply an average spread in the tunnelling distance of approximately 0.3 Å in ferrous myoglobin.

Temperature dependence of $\langle x^2 \rangle_{cv}$

Additional information can be obtained from the temperature dependence of $\langle x^2 \rangle_{cv}$. We simplify the situation shown in Fig. 1 by assuming that each atom moves in a conformational potential, V_v , indicated by dashed lines. We approximate the isotropic potential, $V_v(x)$, $x > 0$, by a power law

$$V_v(x) = \text{const. } x^{1/\nu} \quad (6)$$

Three different potentials are given in Fig. 1. At a temperature $T \gg T_i$, substates at distance x are populated according to a Boltzmann distribution

$$P_v(x; T) \propto x^2 \exp(-\text{const. } x^{1/\nu}/T) \quad (7)$$

The mean-square displacement for $T \gg T_i$ is given by

$$\langle x^2 \rangle_v = \int dx x^2 P_v(x; T) / \int dx P_v(x; T) = \text{const. } T^{2\nu} \quad (8)$$

where the constant depends on ν but not on T . We set $\langle x^2 \rangle_v = \langle x^2 \rangle_{cv}$ and obtain ν from plots of $\log \langle x^2 \rangle_{cv}$ against $\log T$. The values for the backbone (ν_b) and the side chain (ν_s) are given in Fig. 2d and e. Residues with small $\langle x^2 \rangle_{cv}$ generally have large values of ν , those with large $\langle x^2 \rangle_{cv}$ small values. Averaged over atoms with the same value of $\langle x^2 \rangle_{cv}$, the exponent ν satisfies the relation

$$\nu = \nu_0 \exp(-\xi \langle x^2 \rangle_{cv}) \quad (9)$$

where $\nu_0 = 4$, $\xi = 22 \text{ Å}^{-2}$ and with $\langle x^2 \rangle_{cv}$ measured at 250 K. Regions of the protein with small displacements behave like solids and have steep potential wells (V_s in Fig. 1). In contrast, most parts with large $\langle x^2 \rangle_{cv}$ move in broad square wells (like $V_{0.05}$ in Fig. 1) and thus have many substates of about equal energy³³. Only a few residues seem to have harmonic potentials, $\nu = 1/2$.

The behaviour of two residues, Arg 31 and Thr 67, is shown in Fig. 4. Figure 4a displays $\langle x^2 \rangle_{cv}$ for some atoms of the main chain and all non-hydrogen atoms of the side chains. For Arg 31, which lies on the outside of the protein, the displacements are large and increase with distance from the main chain. The displacements for Thr 67, which is in contact with the haem, are much smaller and show no clear dependence on distance. In Fig. 4b, $\langle x^2 \rangle_{cv}$ for some atoms of the two residues is plotted against temperature. The displacements of the atoms of the side chain of Arg 31 are essentially temperature independent ($\nu < 0.1$), whereas the atoms of Thr 67 show a pronounced temperature dependence ($\nu > 3$). The standard deviation of $\langle x^2 \rangle_{cv}$, 0.015 Å², is shown on some of the data points.

Figure 2a and b contains only one-fifth of our data at one temperature. Plotting all data would convey more information, but also obscure essential features. However, computer graphics provide a means of spatial representation of our results. Atoms are drawn as spheres with van der Waals radii and placed at their mean positions. Each atom is shaded according to the displacement $\langle x^2 \rangle_{cv}$. The protein is then cut in the desired plane, atoms exposed by the cut become visible, and structure and motion can then be observed at the same time. Figure 5 displays two such cuts through myoglobin at 250 K. A condensed core around the active centre, semi-liquid outer shells, and at least one pathway to the pocket on the distal side of the haem are apparent. The core can probably be likened to an aperiodic solid: the number of conformational substates per group of atoms is small, $\langle x^2 \rangle_{cv}$ is less than 0.04 Å² and the temperature coefficient ν is larger than 2. Residues in the semi-liquid regions have larger conformational displacements $\langle x^2 \rangle_{cv} \sim 0.04$ – 0.25 Å^2 , and can probably assume many substates, and ν is generally less than 2. The term semi-liquid implies that the structure is liquid-like within volumes of a few Å³; molecules may undergo brownian motion within the volumes accessible to them (micro-brownian motion) and the semi-liquid region may thus be very similar to glasses³⁴. These features agree with the

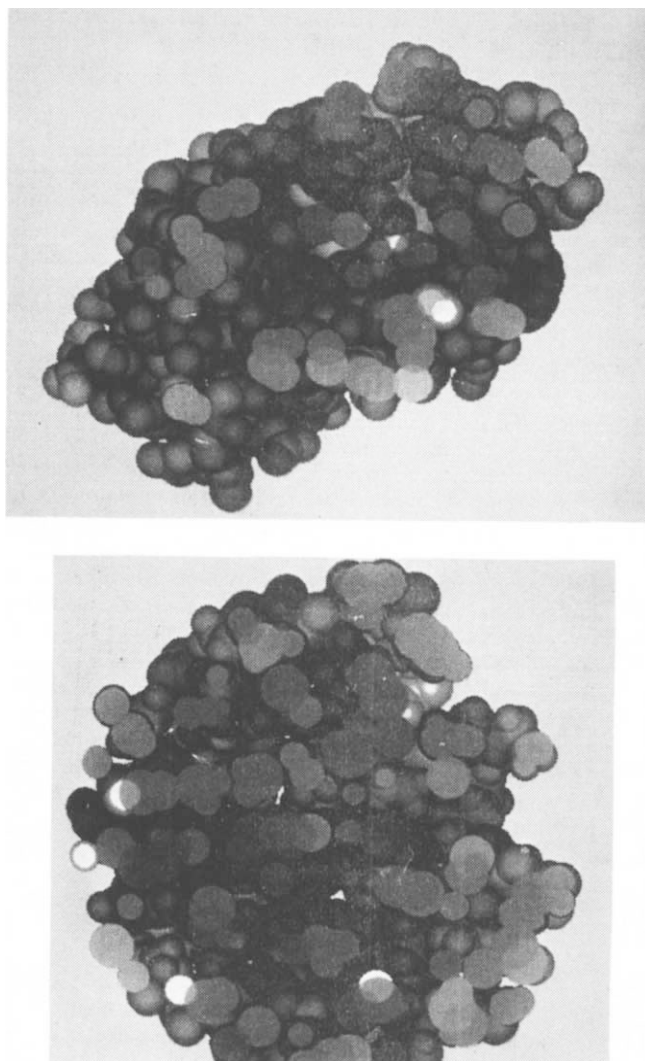


Fig. 5 Two slices through the 'dynamic' structure of myoglobin. Atoms are shown as spheres with van der Waals radii centred at their mean X-ray positions. Spheres interpenetrate when atoms are covalently bonded. The shading of each atom is proportional to the mean-square displacement $\langle x^2 \rangle_{cv}$ at 250 K; the heavier the shading of the atom, the smaller the value of $\langle x^2 \rangle_{cv}$. The upper cut is in a plane parallel to the haem plane and slightly towards the distal histidine side. The lower cut is at right angles to the haem plane and passes through residues 93 and 106; only the 'active site' area is shown. The haem plane is horizontal in the centre of the figure; Arg 45 is to the right.

description used by McCammon, Gelin and Karplus ("fluid-like in that the local atom motions have a diffusional character")¹⁵. The displacement $\langle x^2 \rangle_{cv}$ increases nearly continuously from the inside to the protein surface.

Future prospects

Our results suggest that the extraction of $\langle x^2 \rangle$ from the intensity of the diffraction peaks should be carried out for non-isotropic distributions (assumption (1)) and for charge densities that are not gaussian (assumption (2))³⁵. In protein structure refinement the standard method assumes correct bond lengths and bond angles, and no poor van der Waals contacts. The average structure, which fits the observed X-ray data best, will have apparent wrong bond lengths and angles and poor van der Waals contacts.

X-ray diffraction yields only information about the spatial distribution of protein motion. The temporal behaviour must be studied by other means, such as NMR, fluorescence relaxation, flash photolysis and molecular dynamics³⁶. The results present-

ted in Figs 2–5 may provide the maps to guide such work. As some of these other tools may be applied to solutions and single crystals, they may also answer the question of whether the dynamic properties of a protein are identical in a crystal and a solvent.

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1. Careri, G. in *Quantum Statistical Mechanics in the Natural Sciences* (eds Kursunoglu, B., Mintz, S. L. & Widmayer, S. M.) 15–35 (Plenum, New York, 1974).
2. Careri, G., Fasella, P. & Gratton, E. *Crit. Rev. Biochem.* **3**, 141–164 (1975).
3. Austin, R. H., Beeson, K. W., Eisenstein, L., Frauenfelder, H. & Gunsalus, I. C. *Biochemistry* **14**, 5355–5373 (1975).
4. Alberding, N. *et al. Biochemistry* **17**, 43–51 (1978).
5. Lakowicz, J. R. & Weber, G. *Biochemistry* **12**, 4171–4179 (1973).
6. Munro, I. M., Pecht, I. & Stryer, L. *Proc. natn. Acad. Sci. U.S.A.* **76**, 56–60 (1979).
7. Jones, W. C., Jr, Rothgeb, T. M. & Gurd, F. R. N. *J. biol. Chem.* **251**, 7452–7460 (1976).
8. Wüthrich, K. & Wagner, G. *Trends biochem. Sci.* **3**, 227–230 (1978); *Nature* **275**, 247–248 (1978).
9. Williams, R. J. P. *Proc. R. Soc. B200*, 353–389 (1978).
10. Englander, S. W., Downer, N. W. & Teitelbaum, H. A. *Rev. Biochem.* **41**, 903–924 (1972).
11. Woodward, C. K. & Rosenberg, A. *J. biol. Chem.* **246**, 4105–4113 (1975).
12. Landau, L. D. & Lifshitz, E. M. *Statistical Physics* (Pergamon, London, 1958).
13. Cooper, A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2740–2741 (1976).
14. Lifshitz, I. M. *Soviet Phys. JEPT* **28**, 1280–1286 (1969).
15. McCammon, J. A., Gelin, B. R. & Karplus, M. *Nature* **267**, 585–590 (1977).
16. Willis, B. T. M. & Pryor, A. W. *Thermal Vibrations in Crystallography* (Cambridge University Press, 1975).
17. Cyvin, S. J. *Molecular Vibrations and Mean Square Amplitudes* (Elsevier, Amsterdam, 1968).

18. Lipkin, H. J. *Ann. Phys.* **26**, 115–121 (1964).
19. Dash, J. G., Johnson, D. P. & Visscher, W. M. *Phys. Rev.* **168**, 1087–1094 (1968).
20. Singwi, K. S. & Sjolander, A. *Phys. Rev.* **120**, 1093–1102 (1960).
21. Douzou, P., Hui Bon Hoa, G. & Petsko, G. A. *J. molec. Biol.* **96**, 367–380 (1975).
22. Petsko, G. A. *J. molec. Biol.* **96**, 381–392 (1975).
23. Wyckoff, H. W. *et al. J. molec. Biol.* **27**, 563–578 (1967).
24. North, A. C. T., Phillips, D. C. & Mathews, F. S. *Acta crystallogr.* **A24**, 351–359 (1968).
25. Konnert, J. H. *Acta crystallogr.* **A32**, 614–617 (1976).
26. Takano, T. *J. molec. Biol.* **110**, 537–568 (1977).
27. Parak, F. & Formanek, H. *Acta crystallogr.* **A27**, 573–578 (1971).
28. Dwivedi, A., Pederson, T. & Debrunner, P. G. *Proc. Mössbauer Conf.*, Kyoto (1978).
29. Sternberg, M. J. E., Grace, D. E. P. & Phillips, D. C. *J. molec. Biol.* **130**, 231–253 (1979).
30. Artymiuk, P. J. *et al. Nature* **280**, 563–568 (1979).
31. Perutz, M. F. *Scient. Am.* **239**, No. 6, 92–125 (1978).
32. Frauenfelder, H. in *Tunneling in Biological Systems* (eds B. Chance *et al.*) (Academic, New York, in the press).
33. Gavish, B. *Biophys. Struct. Mech.* **4**, 37–52 (1978).
34. Saito, N., Okano, N., Iwayanagi, S. & Hideshima, T. *Solid St. Phys.* **14**, 343–502 (1963).
35. Jensen, L. H. in *Crystallographic Computing Techniques* (ed. F. R. Ahmed) (Munksgaard, Copenhagen, 1976).
36. McCammon, J. A., Wolynes, P. G. & Karplus, M. *Biochemistry* **18**, 927–942 (1979).
37. Dayhoff, M. O. (ed.) *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Silver Spring, 1972).

Crystallographic studies of the dynamic properties of lysozyme

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The patterns of atomic displacements in the crystals of hen and human lysozyme derived from independent crystallographic refinement are broadly similar. Analysis of the pattern indicates a close correlation with molecular structure, strongly suggestive of intramolecular motion. The active site of lysozyme is located in a region of high displacement. It is concluded that protein mobility may play a significant part in biological activity and that X-ray crystallography can contribute to its analysis.

THE distribution of electron density within a molecule, which is given by X-ray analysis of single crystals, can be used to determine two major pieces of information about the atoms making up the molecule. First, the mean positional coordinates of each atom can be obtained from the maxima in the distribution, and second, the mean square amplitude of displacement of each atom from its mean position can be derived from the rate at which the electron density falls off from the corresponding maximum. A quantitative estimate of the mean square displacement can be obtained from crystallographic refinement in reciprocal space in which the electron density of each atom is modelled by $f \exp(-Bs)$, where f is the scattering factor for the atom at rest, s is a reciprocal lattice vector, and B (often called the temperature factor) is related to the mean square amplitude of harmonic displacement (U^2) by the expression $B = 8\pi^2 U^2$. If

isotropic displacement is assumed, the B -factor has a single value; however, anisotropic displacement can be modelled by splitting B into six parameters whose values define the axial lengths and orientation of an ellipsoid of displacement.

Although analysis of B -factors is routinely used in the crystallography of small molecules¹, it is much less frequently applied to protein molecules, despite the possible significance^{2–4} of knowledge of atomic displacements for the study of protein dynamics, and their role in biological activity. At least part of the reason for this is that there are several experimental difficulties in obtaining accurate B values, and theoretical problems in relating observed displacements to true atomic motion, which are exacerbated in the case of very large molecules like proteins. The most vexing of the theoretical problems arises because the X-ray measurements are averaged over time and, in effect, over crystal space. Consequently, there is no time component associated with the observed displacements, and this makes it impossible to distinguish directly between true atomic motion derived from vibrational and rotational modes, and static disorder caused by different molecules in the crystal adopting different conformations, or occupying different positions and orientations in the various unit cells. A possible way of distinguishing true atomic motion from static disorder is by analysis of X-ray intensities collected at different temperatures⁵. (See accompanying paper⁶ for a discussion of this, and other approaches to the problem.)

At least two other problems at this level of significance must be considered before any attempt can be made to use B factors to describe protein dynamics. First, because the values of the B factors are determined largely from the variation of the X-ray

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pattern with diffraction angle, the observed displacements are potentially very sensitive to systematic errors in the collection and correction of the X-ray measurements. Errors of this kind are particularly difficult to avoid when using large⁷, liquid-containing, radiation-unstable⁸ protein crystals. In addition, observable X-ray intensities from protein crystals are restricted to lower diffraction angles than those from most crystals of small molecules; this will therefore reduce the precision of the observed displacements. Second, the crystal packing may modify the atomic displacements as compared with those in free solution. Unlike small molecules, globular protein molecules are stabilised by the same weak forces that stabilise the crystals, and, although this factor may endow protein molecules with a richer variety of motions than small molecules, it may also increase the relative influence of the crystal environment on the actual displacements.

In this report we compare the displacements of the homologous hen and human lysozyme molecules, which crystallise in quite different space groups. Our results were derived from independent refinements of sets of X-ray intensities at different resolution, which were collected and corrected by different procedures. The close agreement of the displacements not only suggests that the effects of experimental error and crystal packing are not as serious as might be thought, but also that the pattern of displacements may represent a characteristic molecular property of biological significance.

Treatment of X-ray intensity data

The values of the B factors are derived from independent refinements of hen egg-white lysozyme (HEWL) at 2 Å resolution (D.E.P.G. and D.C.P., unpublished) and the homologous human lysozyme (HL) at 1.5 Å resolution (P.J.A. and C.C.F.B., unpublished). The two enzymes crystallise in unrelated space

groups, HEWL in $P4_32_12$ with $a = b = 79.1$ Å, $c = 37.9$ Å (ref. 10), and HL in $P2_12_12_1$ with $a = 57.1$ Å, $b = 61.0$ Å, $c = 32.9$ Å (ref. 14). In their respective unit cells the molecules are quite differently packed.

The collection and correction of the X-ray intensity data were carried out using different procedures. The X-ray intensities of HEWL¹⁰ were collected in levels in reciprocal space on a linear diffractometer adapted to measure three reflections simultaneously¹¹. More than half the reflections were measured four or more times and fewer than 5% only once. No crystal was used for longer than 20 h so that the unreduced data comprised ~30,000 measurements from 14 crystals. In contrast, the intensities of HL were measured on a computer-controlled five-circle diffractometer equipped to measure five reflections simultaneously¹². The intensities were collected in shells in reciprocal space, each reflection being measured at least twice. Only one crystal was used for the complete data set of 50,000 reflections.

Data reduction and correction were also carried out by different methods: the net intensities of HEWL were obtained by the background-peak-background method, those of HL by profile fitting¹³. Radiation damage in HEWL was estimated from a common row of reflections measured before and after each level, and the correction was applied to each triplet level. In HL¹⁴ it was estimated from a set of reference reflections monitored throughout the data run, and applied as a single correction to the whole data set. Absorption was corrected in HEWL by a two-dimensional⁷, and in HL by a three-dimensional method¹⁵.

Refinement of the HEWL structure was carried out by automated shifts estimated from difference syntheses¹⁷, using the RSSD coordinates produced by the Diamond real-space refinement¹⁶ as the starting point. Constraints on covalent bond lengths and angles, but not on non-bonded or hydrogen-bond interactions, were applied to the coordinates after one or more

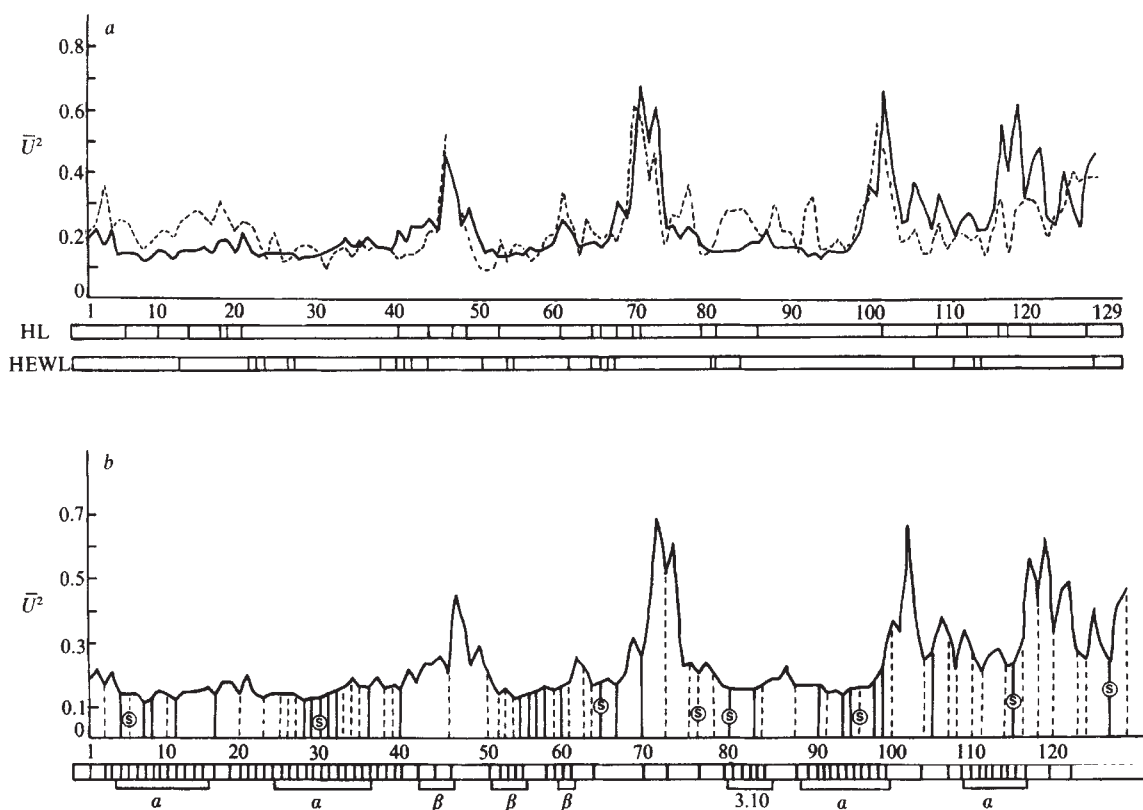


Fig. 1 *a*, A plot of mean main-chain $\overline{U}^2$ values against residue number for HL (full line) and HEWL (broken line). The numbering scheme is for HEWL; the additional residue in HL is inserted between residues 47 and 48. Residues involved in intermolecular contacts in the crystals of the two molecules are indicated in the lower rectangles. *b*, As *a* for HL. The full vertical lines indicate the location of internal residues in the lysozyme molecule, and the broken vertical lines residues lying in the molecular surface²⁴. The residues involved in intramolecular hydrogen bonds are shown in the lower rectangle.

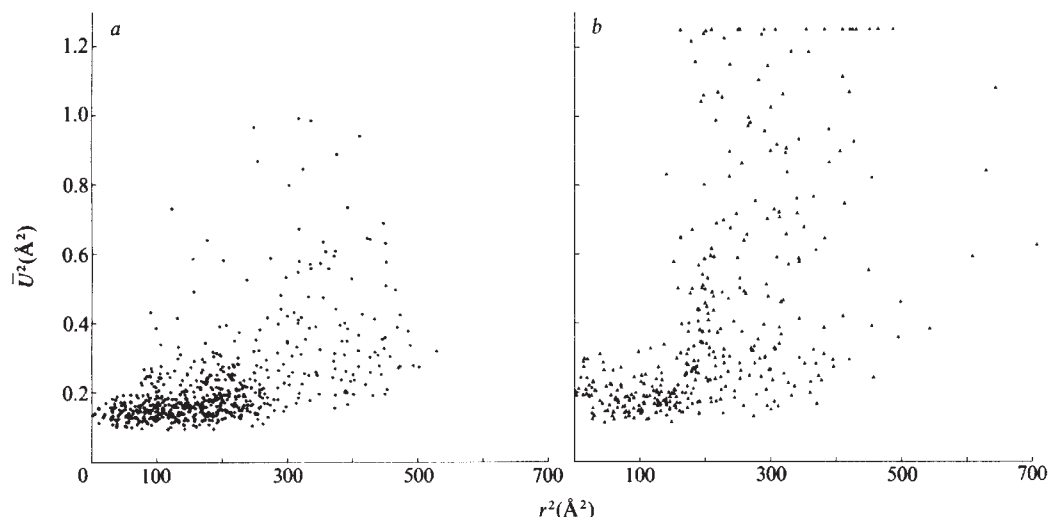


Fig. 2 A plot of atomic $\overline{U}^2$ values of HL against the square of their distance from the molecular centroid. *a*, Main-chain atoms; *b*, side-chain atoms.

cycles of shifts. Because at 2 Å resolution there are only 9,600 structure amplitudes, only two B factors were used to describe each residue, one for the four main-chain atoms and the β -carbon, and another for the side-chain atoms beyond C^β . The refinement has not been completed but the R factor is currently 0.22 for all 9,600 terms using 1,001 protein atoms and some 250 water molecules. The initial stages of the HL refinement also used difference syntheses, but the later stages were mainly carried out using the constrained least-squares method of Hendrickson and Konnert¹⁸. Rather tight constraints were imposed on the covalent structure of the protein: covalent bond lengths were not allowed to vary by more than 0.01 Å. However, the weights of the constraints on non-bonded interactions and hydrogen bonds were reduced to the minimum values allowed by the Konnert programme (10^{-8} of the weight of the covalent constraints), and no constraints were imposed on the B factors at any point. At intervals during the least-squares refinement, a few cycles of difference map shifts were carried out to monitor the course of the least-squares refinement. The additional resolution available for HL allowed refinement of individual B factor for each atom. The current R factor for all 19,000 terms to 1.5 Å is 0.18 (≈ 0.17 at 2 Å resolution) using 1,026 protein atoms and 130 water molecules.

We know that the use of tight stereochemical constraints may not always be appropriate because of the effects of libration, first discussed by Cruickshank³³. On the other hand, because constraints are used to compensate for the lack of true atomic resolution even at 1.5 Å, relaxation of the constraints to allow for libration would be expected to result in some degree of randomisation of the atomic positions elsewhere. Preliminary consideration of this complex problem suggests that the B values of atoms positioned incorrectly, through the application of inappropriate constraints or any other factor, will become anomalously high so as to maximise the overlap between the model and the real electron density.

Comparison of the displacements

Figure 1a shows a plot of the mean square displacement of the main-chain atoms of HEWL and HL against residue number. For HEWL, $\overline{U}^2$ has been obtained from the common B factor for the N, C $^\alpha$, C', O and C $^\beta$ atoms for each residue, and for HL it has been obtained by calculating the mean B value for the equivalent five atoms in each residue. It can be seen that, to a first approximation at least, the two plots have the same characteristics: a fairly constant background on which are superposed several peaks corresponding to residues 47, 70–73, and 101 and 102. Although there are some differences, particularly around residues 117–119 and 121 and 122, which in HL have greater displacements than in HEWL, the general agreement is good, as shown by the correlation coefficient of 0.61. The distribution of

$\overline{U}^2$ is much smoother for HL, which almost certainly reflects the more accurate B factors obtained from the more complete refinement of higher resolution data. As each step in the collection, reduction and correction of the X-ray data from the two molecules has been carried out differently, it is most unlikely that the parallel variations in these curves represent merely the incorporation of systematic experimental error into the B values. Similarly, because the two enzymes are packed together in their respective crystal lattices in quite different ways, the observed variations in $\overline{U}^2$ cannot in the main be due to interactions in the crystal.

These considerations suggest that the parallel variation of the displacement curves represents some particular property of the HEWL and HL molecules themselves. As there are 51 side-chain substitutions and one additional residue in the HL molecule^{19,20} as compared with HEWL, it seems probable that the main chain is the major determinant of the variation. We will now consider two major possibilities: (1) that the variation is a consequence of a rigid-body displacement or simple intramolecular displacement, and (2) that it corresponds to more complex intramolecular displacements. Broadly, these two possibilities can be investigated by determining whether the displacements are related to the shape of the molecule, or to its structure, respectively.

Analysis of main-chain displacements

Various models of possible overall intermolecular and intramolecular displacement of the averaged main-chain $\overline{U}^2$ values were examined. All the models that M.J.E.S., D.E.P.G. and D.C.P.²¹ describe in their analysis of HEWL were considered for both molecules, and the salient results are summarised below. The best parameters to describe each model were found by a least-squares fit to the observed $\overline{U}^2$ values, and the quality of fit by a parameter R^2 , expressed by

$$R^2 = \frac{\sum_{a=1}^N [(\overline{U}^2_a)_{\text{obs}} - (\overline{U}^2_a)_{\text{calc}}]^2}{\sum_{a=1}^N [(\overline{U}^2_a)_{\text{obs}} - \overline{U}^2_{\text{mean}}]^2}$$

where

$$\overline{U}^2_{\text{mean}} = \frac{\sum_{a=1}^N (\overline{U}^2_a)_{\text{obs}}}{N}$$

and N is the number of residues. $R^2 = 0$ indicates total agreement and $R^2 = 1$ the worst possible agreement. The models described are as follows.

Model A: a rigid body mode, consisting of a uniform translational displacement together with independent librational displacements about three principal axes that intersect at the centroid (the 'TL' model of Cruickshank²²).

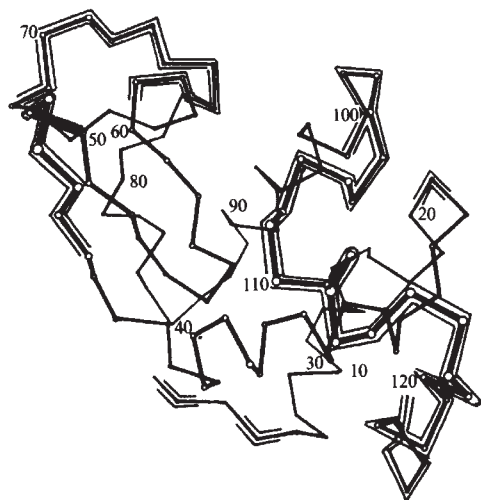


Fig. 3 A perspective drawing of the lysozyme molecule with the residues having displacement $>0.2 \text{ \AA}^2$ in HL outlined by the parallel lines. The active site cleft runs almost vertically down the front of the drawing.

Model B: a general 'breathing' mode, which is a reinterpretation of model A as intramolecular displacements consisting of a uniform component together with displacements that increase with distance from the molecular centroid, and have independent magnitudes along the three principal axes.

Model C: an intramolecular 'hinge bending' mode, in which displacements corresponding to a variation in the separation of the two lobes of the molecule that enclose the active site cleft, are superimposed on a uniform displacement. This mode is analogous to that studied by Karplus and coworkers²³ using energy calculations of molecular dynamics.

In neither molecule did hinge bending account for much of the variation [$R^2(\text{HEWL}) = 0.79$; $R^2(\text{HL}) = 0.79$], even though, as shown below, the lips of the active-site cleft correspond to regions of high displacement. Rigid-body displacement (model A) or the equivalent breathing mode (model B) gave better agreement [$R^2(\text{HEWL}) = 0.54$; $R^2(\text{HL}) = 0.49$]. The magnitudes of the libration or breathing for each molecule were significantly, and similarly, non-uniform along the three principal axes. However, the directions of the axes of principal displacement in HEWL and HL differed by 35° . Certain residues, including those marking the peaks in Fig. 1a, had markedly higher observed U^2 values than were accounted for by models A and B, and these residues probably have additional intramolecular displacements. Models A and B were therefore amended to include and determine the additional motion of each of these residues, increasing the agreement of these models to $R^2(\text{HEWL}) = 0.17$ and $R^2(\text{HL}) = 0.13$. Without anisotropic B values it is difficult to distinguish between the alternatives of rigid-body displacement and intramolecular breathing.

Given that even for the rigid-body model considerable allowance had to be made for intramolecular displacement, we have examined independently the case for intramolecular displacement by considering the relationship between the U^2 values and the molecular fold in terms of regular secondary structure and the relative exposure of the side chains²⁴. Figure 1b shows the plot of this relationship, which indicates that segments of regular secondary structure, both α and β , correspond quite closely to the relatively smooth minima in the U^2 distribution. Closer examination reveals some correlation with the finer details of structure; for example, there are cyclic variations along the 4–16 and 108–116 helices that correspond to greater displacements of residues located on the exposed surface of these helices, and the most exposed strand, residues 42–46, of the small three-stranded β -sheet in lysozyme has a consistently higher displacement than the other two. There is also a good correlation between local minima in the U^2 plot with

internal or surface residues. This is particularly striking in regions where there is little or no regular secondary structure; for example, in the C-terminal region, from residue 100 onwards, each downward trend in the sawtooth distribution of displacement corresponds to an internal or surface residue, and two of the deepest troughs correspond to the two disulphide links in this region, residues 115 and 127. Thus, the regions of high displacement correspond to main-chain segments that are not involved in regular secondary structure and whose side chains are external. These close correlations of displacement with structure reinforce the conclusion that we are observing a characteristic molecular property, and not some artefact of the crystallography.

If the regions of high displacement ($\overline{U^2} > 0.2 \text{ \AA}^2$) shown in Fig. 1a for HL are located on the folded lysozyme molecule (Fig. 3), it can be seen that most of them will lie on a continuous strip on the enzyme surface that begins at the exposed strand of the β -sheet, runs along to the 'top' of the molecule, down one side of the active-site cleft and along to the C-terminus. It is intriguing that this region of high displacement includes both lips of the active-site cleft³¹, which is also the principal antigenic determinant of lysozyme³², and also most of the main-chain segments which undergo conformational change when ligands are bound³¹.

Analysis of side-chain displacements

Figure 2b shows a plot of $\overline{U^2}$ against r^2 , where r is the distance from the molecular centroid for the side chains of human lysozyme. The striking feature of this plot is the clear discontinuity in the U^2 distribution at an r^2 value of about $180 \text{ \AA}^2$: below this value the displacements are closely grouped between 0.1 and $0.3 \text{ \AA}^2$, but above this value the displacements vary widely, with several displacements up to $1.25 \text{ \AA}^2$ corresponding to the arbitrary allowed maximum for B factor of $100 \text{ \AA}^2$. The lysozyme molecule is ellipsoidal, with dimensions of about $30 \text{ \AA} \times 30 \text{ \AA} \times 45 \text{ \AA}$ (ref. 10). Thus, the value of r at the discontinuity of about $14 \text{ \AA}$ corresponds quite closely to the minimum radius of the molecule. This shows that the internal residues, those with $r^2 < 180 \text{ \AA}^2$, are subject to quite small displacements of the same order as the main-chain displacement in this region (Fig. 2a), whereas those side chains with $r^2 > 180 \text{ \AA}^2$, which are mostly external, have widely varying displacements, some of which are very large. To analyse the pattern of side-chain displacements more closely we have listed the mean difference between the side-chain and main-chain displacement for each type of amino acid residue (Table 1). With the exception of the smallest of the hydrophobic residues (Ala) all types of side chains are displaced more than their local main

Table 1 Incremental displacements of the side chains of human lysozyme

Type	No.	$\langle \Delta \overline{U^2} \rangle$	Type	No.	$\langle \Delta \overline{U^2} \rangle$
Ala	14	-0.006 ± 0.020	Ser	6	0.024 ± 0.052
Cys	8	0.008 ± 0.028	Thr	5	0.013 ± 0.055
Val	9	0.012 ± 0.057	Pro	2	0.145 ± 0.173
Ile	5	0.023 ± 0.019	Asp	8	0.152 ± 0.079
Leu	8	0.069 ± 0.043	Asn	10	0.208 ± 0.207
Met	2	0.051 ± 0.030	His	1	0.404
Phe	2	0.051 ± 0.046	Tyr	6	0.108 ± 0.157
Trp	5	0.049 ± 0.027	Glu	3	0.380 ± 0.351
			Gln	6	0.387 ± 0.280
			Lys	5	0.251 ± 0.230
			Arg	14	0.380 ± 0.276

$\langle \Delta \overline{U^2} \rangle = (\sum_{a=1}^n \langle \overline{U^2}_a \rangle \text{ side-chain atoms} - \langle \overline{U^2}_a \rangle \text{ main-chain atoms})/n$, where n is the number of side chains of a given type. In the calculation the β -carbon is considered to be part of the main chain: relaxation of this condition is required to calculate the incremental displacement for alanines. The increase of the spread of the values with increasing $\langle \Delta \overline{U^2} \rangle$ shows that the side chains with largest incremental displacements also show the largest variations.

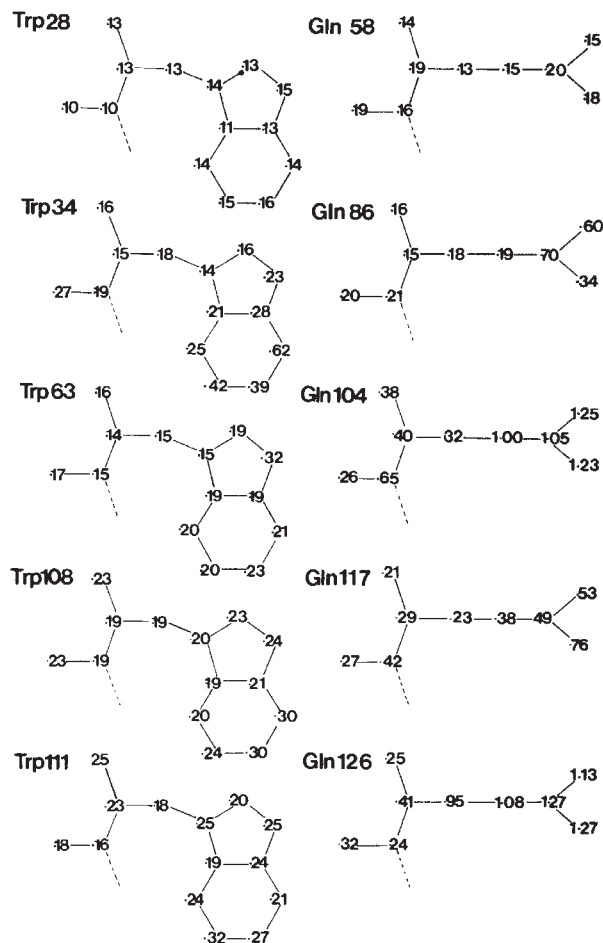


Fig. 4 The five tryptophan residues, and five of the six glutamine residues of human lysozyme showing the individual mean-square displacements of their atoms in $\text{\AA}^2$. Trp 28 is the only residue of this group that is totally buried in the core of the molecule, and its atoms show consistently low displacements. In Trp 34, 108 and 111 it can be seen that the atoms of the six-membered part of the indole ring have larger displacements than the other atoms, consistent with some degree of 'flapping' displacement about their α - β or β - γ bonds. In contrast to the tryptophan residues, the glutamine residues, which with the exception of Gln 58 are external, show increasingly large displacements along their length. Gln 58 lies in the surface of the enzyme with its amide group hydrogen bonded to the main chain, and the displacements of its atoms are lower and do not increase along the length of the side chain. The pattern of displacements in other types of side chain are fully consistent with these examples.

chains. The positive increment is quite small for all hydrophobic residues, but increases sharply with length for the hydrophilic side chains. This analysis reinforces the above conclusion, and suggests that the displacement of side chains is governed largely by their environment and their length, or perhaps, more precisely, by the number of bonds about which free rotation is possible. Figure 4 shows the atomic displacements of the tryptophan and glutamine residues. We have also made a preliminary analysis of anisotropic displacements of certain side chains. Figure 5 shows the results for Trp 63 which suggest that the indole ring of this residue undergoes greatest displacement in the plane of the ring.

Discussion

This study suggests that X-ray crystallography can be used to describe the atomic displacements in a protein molecule. Preliminary analyses indicate that the displacements must have an intramolecular component superimposed on either a rigid-body motion or a general breathing mode. The close correlation

between the displacements and the molecular structure suggests that the observed pattern of displacements is characteristic of the lysozyme molecule. Possibly the most significant finding is that the lips of the active-site cleft, and the region of the enzyme that undergoes conformational change on ligand binding to the active site, are included in the regions that have the greatest displacements.

Unfortunately, the X-ray data described here do not allow us to interpret these displacements directly in terms of motion. There is, however, both theoretical and experimental evidence of intramolecular motion in proteins. Scheringer²⁵ has shown that even in molecules much smaller than lysozyme, the contribution of intramolecular motion increases with the number of atoms in the molecule. His analysis gives a rough estimate of the mean-square amplitude of intramolecular motion in lysozyme as $0.2 \text{ \AA}^2$, which compares remarkably well with mean-square displacement amplitude of $0.23 \text{ \AA}^2$ that we observe for the main-chain atoms of both HEWL and HL. A study of the dynamic motion of the pancreatic trypsin inhibitor⁴ by energy calculations has estimated an r.m.s. displacement for all non-hydrogen atoms of $0.9 \text{ \AA}$, the main chain moving less than the side chains. Nuclear magnetic resonance studies of HEWL in solution²⁶⁻²⁸ have shown that Tyr and Phe residues 'flip' by 180° about their β - γ bonds; this requires considerable movement in neighbouring atoms and that external side chains undergo a rapid waving motion. Hydrogen-isotope exchange data for proteins have been interpreted as indicating breathing motions in proteins²⁹, and the similarity in the rates of hydrogen-tritium exchange of HEWL in solution and in the crystal³⁰ implies that the motion is not reduced by crystallisation.

This evidence of motion in proteins, which is consistent in a qualitative sense with our observations, suggests that it is reasonable to allocate some part of the observed displacement to molecular motion. However, there must be further investigation of methods, such as the analysis of the temperature dependence of the displacements, that may help in apportioning the relative contributions of static disorder and motion to the observed displacements. The analysis of the motion in terms of the contributions from rigid-body and intramolecular modes would be much improved if a complete set of anisotropic B factors could be calculated; this may be possible at $1.5 \text{ \AA}$ resolution or better. It is also evident that thermal diffuse scattering can be observed from proteins using synchrotron radiation (L. N. Johnson, personal communication), which could provide evidence of dominant libration axes¹. The importance of anharmonic vibrations²¹ might also be assessed, for example, by reformulation of the temperature factors in the manner proposed by Dawson³⁴ and Johnson³⁵. Finally, in view of the location of some parts of the active site of lysozyme in regions of high displacement, it would be very interesting to discover the effect of binding substrate-like ligands on the pattern of displacements.

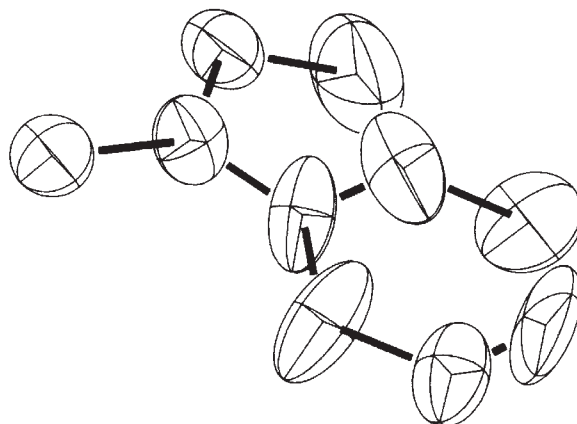


Fig. 5 A conventional drawing of the displacement ellipsoids of Trp 63 obtained from a preliminary analysis of anisotropic B values of the tryptophan residue of human lysozyme.

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1. Willis, B. T. M. & Pryor, A. W. *Thermal Vibrations in Crystallography* (Cambridge University Press, 1975).
2. Blumenfeld, L. A. *J. theor. Biol.* **58**, 269–284 (1976).
3. Ikegami, A. *Biophys. Chem.* **6**, 117–130 (1977).
4. McCammon, J. A., Gelin, B. R. & Karplus, M. *Nature* **267**, 585–590 (1977).
5. Lonsdale, K. & Milledge, J. *Acta crystallogr.* **14**, 59–61 (1961).
6. Frauenfelder, H., Petsko, G. A. & Tsernoglou, D. *Nature* (accompanying paper).
7. North, A. C. T., Phillips, D. C. & Mathews, F. S. *Acta crystallogr.* **A24**, 351–358 (1968).
8. Blake, C. C. F. & Phillips, D. C. in *Biological Effects of Ionising Radiation at the Molecular Level* (IAEA, Vienna, 1962).
9. Blake, C. C. F. & Swan, I. D. A. *Nature new Biol.* **232**, 12–15 (1971).
10. Blake, C. C. F. *et al.* *Nature* **206**, 757–761 (1965).
11. Arndt, U. W., North, A. C. T. & Phillips, D. C. *J. scient. Instrum.* **41**, 421–425 (1964).
12. Banner, D. W., Evans, P. R., Marsh, D. J. & Phillips, D. C. *J. appl. crystallogr.* **10**, 45–51 (1977).
13. French, G. S. thesis, Oxford Univ. (1975).
14. Osserman, E. F., Cole, S. J., Swan, I. D. A. & Blake, C. C. F. *J. molec. Biol.* **46**, 211–212 (1969).
15. Huber, R. & Kopfmann, G. *Acta crystallogr.* **25**, 143–152 (1969).
16. Diamond, R. *J. molec. Biol.* **82**, 371–391 (1974).
17. Oatley, S. J. thesis, Oxford Univ. (1976).
18. Konnert, J. H. *Acta crystallogr.* **A32**, 614–617 (1976).
19. Canfield, R. E., Kammerman, S., Sobel, J. H. & Morgan, F. J. *Nature new Biol.* **232**, 16–18 (1971).
20. Jollès, P. *Chimia (Aarau)* **25**, 1–6 (1971).
21. Sternberg, M. J. E., Grace, D. E. P. & Phillips, D. C. *J. molec. Biol.* **130**, 231–253 (1979).
22. Cruickshank, D. W. J. *Acta crystallogr.* **9**, 754–756 (1956).
23. McCammon, J. A., Gelin, B. R., Karplus, M. & Wolynes, P. G. *Nature* **262**, 325–326 (1976).
24. Imoto, T., Johnson, L. N., North, A. C. T., Phillips, D. C. & Rupley, J. A. in *The Enzymes*, Vol. 7, 3rd edn, Ch. 21 (Academic, London, 1972).
25. Scheringer, C. *Acta crystallogr.* **A28**, 516–522 (1972).
26. Campbell, I. D., Dobson, C. M. & Williams, R. J. P. *Proc. R. Soc. Lond.* **A345**, 41–59 (1975).
27. Dobson, C. M. in *NMR in Biology* (Academic, London, 1977).
28. Blake, C. C. F. *et al.* *Ciba Fdn Symp.* **60**, (1978).
29. Englander, S. W., Downer, N. W. & Teitelbaum, H. A. *Rev. Biochem.* **41**, 903–924 (1972).
30. Praissman, M. & Rupley, J. A. *Biochemistry* **1**, 2446–2452 (1968).
31. Blake, C. C. F. *et al.* *Proc. R. Soc.* **B167**, 378–388 (1967).
32. Atassi, M. Z. & Lee, C.-L. *Biochem. J.* **171**, 429–434 (1978).
33. Cruickshank, D. W. J. *Acta crystallogr.* **9**, 757–759 (1956).
34. Dawson, B. *Proc. R. Soc.* **A298**, 255–263 (1967).
35. Johnson, C. K. *Acta crystallogr.* **A25**, 187–194 (1969).

letters

Soft X-ray emission from the vicinity of MV Lyr

EVIDENCE is presented for the existence of an X-ray source in the range 0.18–0.43 keV at a location consistent with the position of the ‘nova-like’ variable star MV Lyr. The source, designated H1901+43, was observed by the HEAO-1 A2 experiment¹ between 18 and 22 October 1977.

We have analysed data from the smallest field of view of the A2 experiment 0.18–2.5 keV detectors. This has dimensions of 1.55° FWHM in the scan direction (ecliptic latitude) and 3.1° FWHM in the perpendicular direction (ecliptic longitude). The total geometric area of the detector with this field of view is 175 cm². Figure 1 shows the 0.18–0.43 keV scan data in the vicinity of MV Lyr summed over the time interval specified above. A 5σ count excess above background is observed at the latitude of MV Lyr. The solid curve depicts the expected point source response of the detector collimator superposed on a parabolic background function. This combination has been fitted to the data in such a way as to minimise the χ^2 statistic, allowing the strength and position of the source to be free parameters, together with the background terms. Adding higher order terms to the background function does not improve the quality of the fit. The best fit source intensity in the 0.18–0.43 keV band is found to be 1.5 ± 0.3 counts s⁻¹.

No evidence is found for 0.5–2.5 keV emission from H1901+43. However, the data in this band are contaminated by emission from a second harder source, H1918+43, probably associated with the hard X-ray emitter 4U1919+44²(2A1919+435³), which is separated from H1901+43 by ~1° in ecliptic latitude. We can conservatively adopt the measured flux of H1918+43 between 18 and 22 October, 1.0 counts s⁻¹, as an upper limit to 0.5–2.5 keV emission from H1901+43. The constraint thus imposed on the count ratio in the 0.18–0.43 and 0.5–2.5 keV bands limits the temperature of the H1901+43 source to be less than 1.2×10^7 K, assuming an optically thin bremsstrahlung

spectrum. A lower limit to the temperature of ~10⁵ K is implied by the low energy cut-off of the detector. Assuming a spectral temperature of 3×10^5 K, which is consistent with a detection in the 0.18–0.43 keV band only, the observed count rate corresponds to an energy flux of 4×10^{-12} erg cm⁻² s⁻¹ between 0.18 and 0.43 keV.

Figure 2 depicts the two-dimensional error box for H1901+43. The positional bounds in ecliptic latitude have been obtained by fitting the collimator response to the scan data, and correspond to a confidence level of 90%. The source location in ecliptic longitude is defined by the extent of the collimator in this direction (3.1° FWHM) and the daily motion of the satellite as it tracks the Sun. H1901+43 was observed in data taken on several consecutive days and must lie within the common area observed by the collimator on those days. The dimensions of the error box in this direction are therefore less than the full width at base of the collimator and define absolute limits on the position of the source in ecliptic longitude. The error boxes for the 2–10 keV sources 4U1919+46 and 2A1919+438 are also shown in Fig. 2. The positions of these medium energy sources are inconsistent with that of H1901+43.

It can be seen from Fig. 2 that MV Lyr is well contained within the error box of H1901+43. A cursory search revealed no other obvious candidates for the source of the X-ray emission. This, coupled with the fact that several other cataclysmic variables, notably AM Her⁴, U Gem⁵ and SS Cyg⁶ have been found to emit soft X-ray radiation, leads us to suggest MV Lyr as a candidate for identification with H1901+43. Note, however, that the possibility of chance positional coincidence cannot be discounted because of the large positional uncertainty involved. MV Lyr was within the $\pm 3.1^\circ$ longitudinal extent of the detector collimator between 16 and 30 October. H1901+43 was detected in scan data summed between 18 and 22 October, when the mean distance of MV Lyr from the scan path was ~1°. If the identification with the star is correct, the failure to detect X-ray emission after 22 October may imply variability. This would not be surprising in view of the variable nature of the X-ray sources associated with other cataclysmic variables^{4,5}.

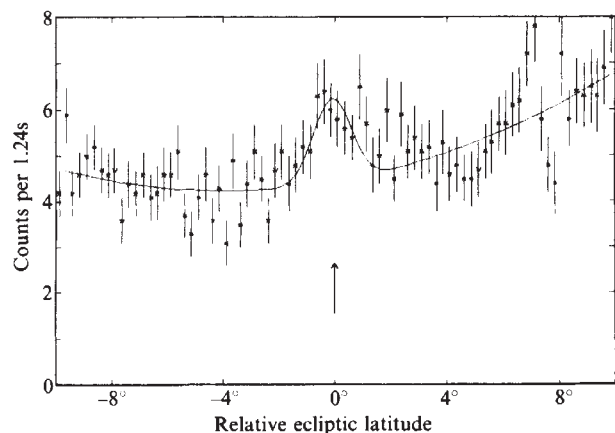


Fig. 1 Summed scan data in the 0.18–0.43 keV band plotted relative to the expected position of MV Lyr (arrow). The solid curve is the best fit collimator response superposed on a parabolic background function.

MV Lyr can be classed among the nova-like stars which have as their prototype UX UMa^{7–9}. It exhibits rapid light variations^{8,10} and a hydrogen emission spectrum which may be superposed on broad Balmer absorption^{8,10–12}. There is evidence that MV Lyr is a binary system with an orbital period of ~ 2 h (ref. 11). The spectrum, luminosity and temporal characteristics of the UX UMa stars are similar to those of the dwarf novae at maximum light, and they may be essentially the same kind of system, but in an extended outburst^{7–9}. Further to this suggestion, we note that if the proposed X-ray identification is correct, the ratio of soft X-ray to optical luminosity for MV Lyr is within a factor of two that observed from U Gem during outburst⁵. The range of absolute magnitude inferred for the UX UMa stars as a class ($3 \leq M_v \leq 6$)⁷ together with the observed visual magnitude of MV Lyr⁷, suggests that this star is at a distance of between ~ 300 pc and $\sim 1,200$ pc, corresponding to an X-ray luminosity range of $(4\text{--}60) \times 10^{31}$ erg s⁻¹. The distance of the star is consistent with its detection at $\sim 1/4$ keV given its relatively high galactic latitude ($b^{\text{II}} = 16^\circ$).

The tentative identification of MV Lyr as a soft X-ray emitter may provide an important clue to the relationship between various types of cataclysmic variable stars. We note that the likely optical counterpart for the X-ray source 2A0526–328 may also be a non-erupting cataclysmic variable¹³. It will be important to seek confirmation of these results, and to search for X-ray emission from other stars in the UX UMa category.

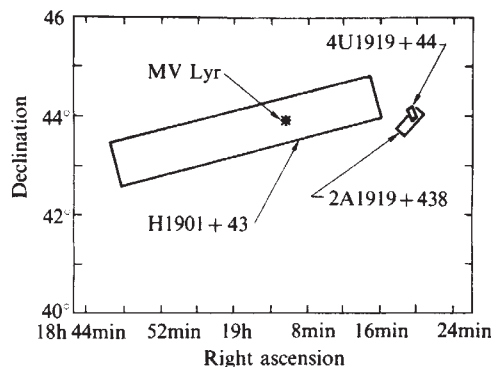


Fig. 2 Error box for H1901 + 43. The confidence level is 90% in the narrow (scan) direction. In the perpendicular direction, the positional uncertainty is defined by the dimensions of the collimator, and the time interval over which the source was detected.

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1. Rothschild, R. E. *et al. Space Sci. Instr.* **4**, 269 (1979).
2. Forman, W. *et al. Astrophys. J. Suppl.* **38**, 357 (1978).
3. Cooke, B. *et al. Mon. Not. R. astr. Soc.* **182**, 455 (1978).
4. Tuohy, I. R., Lamb, F. K., Garmire, G. P. & Mason, K. O. *Astrophys. J. Lett.* **226**, L17 (1978).
5. Mason, K. O., Lampton, M. L., Charles, P. & Bowyer, S. *Astrophys. J. Lett.* **226**, L129 (1978).
6. Rappaport, S., Cash, W., Doxsey, R., McClintock, J. & Moore, G. *Astrophys. J. Lett.* **187**, L5 (1974).
7. Warner, B. *Observatory* **96**, 49 (1976).
8. Warner, B. *IAU Symp. No. 73*, 85 (1977).
9. Robinson, E. L. *A. Rev. Astr. Astrophys.* **14**, 119 (1976).
10. Walker, M. F. *Publ. astr. Soc. Pacif.* **66**, 71 (1954).
11. Walker, M. F. *Adv. Electronics Electron Phys.* **22B**, 761 (1966).
12. Greenstein, J. L. *Publ. astr. Soc. Pacif.* **66**, 79 (1954).
13. Charles, P., Thorstensen, J., Bowyer, S. & Middleditch, J. *Astrophys. J. Lett.* (in the press).

Parity nonconservation and stellar collapse

ACCORDING to the current theory, the evolution of sufficiently massive stars leads eventually to gravitational instability and collapse. After thermonuclear burning has stopped, dense stellar cores become unstable and collapse to form neutron stars (pulsars) or black holes. In both cases energy of the order of $0.1 Mc^2$ is released, mostly in the form of neutrinos and gravitational waves. As the radius of the core decreases, its magnetic field grows (magnetic flux is conserved) and can reach huge values of order $10^{12}\text{--}10^{13}$ G. Even greater azimuthal fields ($\sim 3 \times 10^{15}$ G) can be generated by differential rotation of the core¹. It will be shown here that neutrino emission in the collapse is asymmetric, as a result of parity nonconservation in weak interactions. Neutrinos and antineutrinos are emitted preferentially in the direction parallel to the local magnetic field of the collapsing core.

The basic physical effect here is the generation of neutrino energy flux by magnetic fields in the presence of electrons (and positrons). The magnetic field polarises the electrons, and neutrinos scattering on electrons come off with their momenta correlated with the magnetic field. Clearly, this effect violates reflectional symmetry, as the energy flux is a polar vector and the magnetic field is an axial vector. The neutrino energy flux depends on the details of neutrino–electron scattering and its calculation requires a fully fledged transport theory. However, a crude estimation can be made if we take into account the following general features of electron–neutrino interaction. Neutrinos and antineutrinos are coupled to left-handed electrons and right-handed positrons more strongly than to right-handed electrons and left-handed positrons, the difference in the coupling constant being of the order of the coupling constant itself (assuming that electrons are relativistic, that is $T \geq 10^{10}$ K). The neutrino–electron cross-section is a rapidly growing function of energy, so that the probability of head-on collision is greater than that in the case of a neutrino running after an electron. Assuming for clarity that the magnetic field is directed upwards, it is easily seen that left-handed electrons and right-handed positrons move preferentially upwards, and thus neutrinos and antineutrinos moving down scatter more

frequently than those moving up. (In this order-of-magnitude estimation we disregard the motion of neutrinos in the transverse plane.) The ratio of the corresponding mean free times is of the order of $\tau_{\downarrow}/\tau_{\uparrow} \sim n_{\uparrow}/n_{\downarrow}$ where $n_{\uparrow}$ and $n_{\downarrow}$ are the densities of electrons with spin up and with spin down, respectively. If $\nu_{\uparrow}$ and $\nu_{\downarrow}$ are the densities of neutrinos moving up and moving down, respectively, then in a stationary flux $\nu_{\uparrow}/\tau_{\uparrow} \sim \nu_{\downarrow}/\tau_{\downarrow}$. Assuming for simplicity that the neutrino chemical potential is smaller than the temperature T , the neutrino momentum density $\mathbf{S}$ is of the order of $n_{\nu}T(n_{\uparrow} - n_{\downarrow})/(n_{\uparrow} + n_{\downarrow})$. Here $n_{\nu} = \nu_{\uparrow} + \nu_{\downarrow} \sim T^3$ is the total neutrino density and the system of units in which $c = \hbar = k = 1$ is used here. In the presence of scatterers other than electrons (such as neutrons or nuclei) the expression for $\mathbf{S}$ has to be multiplied by $\alpha \sim \tau_{\text{tot}}/\tau_{\nu e}$, where $\tau_{\nu e}$ and τ_{tot} are neutrino-electron and total neutrino mean free time, respectively. The ratio $(n_{\uparrow} - n_{\downarrow})/(n_{\uparrow} + n_{\downarrow})$ is of the order of $\mu B/\varepsilon$, where ε is the characteristic electron energy, $\varepsilon \sim T$ or $\varepsilon \sim \zeta_e$ (whichever is greater), $\mathbf{B}$ is the magnetic field, ζ_e is the chemical potential of electrons, and μ is the effective electron magnetic moment, $\mu = e/2\varepsilon$. (The latter equation follows from the expression² for electron energy levels in a constant magnetic field, $\varepsilon = (\varepsilon_0 \pm eB)^{1/2}$, where ε_0 is the spin-independent part of the energy.) The neutrino energy flux can now be written as

$$\mathbf{S} \sim \alpha n_{\nu} T \varepsilon^{-1} \mu \mathbf{B} \quad (1)$$

This equation implies that the average momentum per neutrino is of the order of $\alpha \mu \mathbf{B} T/\varepsilon$. Note that neutrino and antineutrino currents in magnetic field have the same direction. A detailed derivation of equation (1) from the Boltzmann equation will be given elsewhere.

To estimate the role of parity-violating effects in stellar collapse, I shall use the results of numerical calculations for conventional supernovae models^{3,4}. These calculations show that at the neutrino photosphere $T < \zeta_e \sim 13$ MeV, and thus $\varepsilon = \zeta_e \sim 13$ MeV. The neutrino transport is dominated by coherent scattering on nuclei, so that $\tau_{\text{tot}} \sim 0.1 \tau_{\nu e}$ and $\alpha \sim 0.1$. The total momentum carried away by neutrinos is of the order of

$$\mathbf{P} \sim \alpha N_{\nu} T \zeta_e^{-1} \mu \mathbf{B}_{\text{av}} \sim \alpha E_{\nu} \zeta_e^{-1} \mu \mathbf{B}_{\text{av}} \quad (2)$$

where $N_{\nu} \sim E_{\nu}/T$ is the total number of emitted neutrinos, E_{ν} is the energy lost in the form of neutrinos, and $\mathbf{B}_{\text{av}}$ is the magnetic field averaged over the neutrino photosphere (the quantities α , ζ_e and $\mathbf{B}_{\text{av}}$ have also to be averaged over the whole period of intensive neutrino emission). The corresponding recoil velocity of the core is

$$\mathbf{v} \sim -\alpha \mu \mathbf{B}_{\text{av}} E_{\nu} / M \zeta_e$$

For the characteristic values $B_{\text{av}} \sim 5 \times 10^{12}$ G, $E_{\nu}/Mc^2 \sim 0.1$ we get $v \sim 5 \times 10^7$ cm s⁻¹. The actual velocities of pulsars are of the order of 10^7 cm s⁻¹. We see that the effect of asymmetric neutrino emission can account only for a small fraction of this value. The effect can be more important in collapse of magnetic stars where one can expect greater values of the magnetic field.

As already mentioned, the azimuthal magnetic field B_{ϕ} can be much greater than B_{av} . Numerical calculations show¹ that the spatial distribution of the azimuthal field can be rather complicated. B_{ϕ} reaches its maximum value ($\sim 10^{15}$ G) in relatively small localised regions of the core. Neutrino fluxes generated by azimuthal magnetic fields can have a role in a redistribution of the angular momentum of the collapsing core or of a hot neutron star. It can be shown that the angular momentum flux at distance r from the rotation axis is of the order of $\mathcal{L}_{\text{B}} \sim \alpha n_{\nu} T \mu \zeta_e^{-1} l_{\nu} r \nabla B_{\phi}$, where l_{ν} is the neutrino mean free path and it is assumed that the characteristic length scale of B_{ϕ} is smaller than r but greater than l_{ν} . An estimation shows that $\mathcal{L}_{\text{B}}$ can be of a comparable magnitude to that of the neutrino angular momentum flux induced by rotation⁵, $\mathcal{L}_{\text{R}}$. However, the directions and thus the effects of the two fluxes are quite different. $\mathcal{L}_{\text{R}}$ transports angular momentum from the inner to the outer parts of the core. $\mathcal{L}_{\text{B}}$ tends to slow down the rotation in the regions of most intensive azimuthal field if the rotational velocity v_{ϕ} has the same sign as B_{ϕ} and tends to accelerate the rotation if $v_{\phi} B_{\phi} < 0$. If, for clarity

the angular momentum of the core is directed upwards, then B_{ϕ} tends to have opposite signs in upper and lower hemispheres, and thus the flux $\mathcal{L}_{\text{B}}$ develops an asymmetry in the angular velocity distribution.

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1. Le Blanc, J. M. & Wilson, J. R. *Astrophys. J.* **161**, 541-551 (1970).
2. Berestetskii, V. B., Lifshitz, E. M. & Pitaerskii, L. P. *Relativistic Quantum Theory* (Pergamon, New York, 1971).
3. Azneit, W. D. *Astrophys. J.* **218**, 815-833 (1977).
4. Nadyozhin, D. K. *Astrophys. Space Sci.* **53**, 131-153 (1978).
5. Kazanas, D. *Nature* **267**, 501-502 (1977).

Solar radio spectrum extended to 10 km wavelength

KNOWLEDGE of the range of solar radio burst flux densities is important for understanding the physical processes generating these bursts. However, past summaries of the solar radio spectrum^{1,2}, compiled from ground-based observations, have not extended to wavelengths much below 10 m because the ionosphere of the Earth is opaque to longer wavelengths. Consequently, radio waves generated at altitudes above 5-10 solar radii in the outer corona can be observed only from above the ionosphere. The spectrum presented here covers wavelengths of 1 mm to 10 km; the observations between wavelengths of 100 m and 10 km were made by Earth satellites.

At hectometric and kilometric wavelengths thermal radiation from the Sun is typically five orders of magnitude weaker than the experiment threshold sensitivity, determined by local plasma noise and cosmic background noise. Nevertheless, satellites have detected hundreds of thousands of hectometric and kilometric solar radio bursts of nonthermal emission in the past decade. Type III solar radio bursts are by far the most common variety observed at these long wavelengths. According to our present knowledge³, type III bursts are generated when energetic (10-100 keV) electrons are ejected from the Sun and travel outwards along open magnetic field lines. These energetic electrons excite emission at the second harmonic of the local solar wind electron plasma frequency. As the exciter electrons move away from the Sun into regions of lower density, the radiation occurs at progressively longer wavelengths and later times. Average type III burst emission wavelengths are 1 km at 0.3 AU and 5 km at 1 AU. A few type II bursts at wavelengths longer than 100 m have also been observed, occurring at both the fundamental and second harmonic of the plasma frequency in association with a propagating interplanetary shock^{4,5}.

Figure 1 includes long wavelength data from the Interplanetary Monitoring Platform IMP6 and the Radio Astronomy Explorer RAE1 Earth orbiters. Radio astronomy experiments on these satellites use long wire antennas, 91 m and 229 m respectively, connected to multi-channel radiometers in the frequency range of 25 kHz to 10 MHz. The IMP6 orbital altitude ranged from 2 to 33 Earth radii (the experimental arrangement has been described by Brown⁶). RAE1 operated in a circular orbit at an altitude of one Earth radius (its experiment systems have been discussed in detail elsewhere⁷).

Flux density measurements for very intense bursts are shown in Fig. 1. Smaller bursts are much more numerous. The distribution of burst maximum intensities at a given wavelength follows a power law, $n = A(\lambda)S^{-\beta}$, where n is the occurrence rate (bursts per day per unit flux density), S is the observed flux density, and $A(\lambda)$ is a constant for each wavelength. Observations demonstrate that $\beta \approx 1.5$ over a wavelength range of 0.1-300 m⁸⁻¹⁰. Accordingly, for a given wavelength, there are

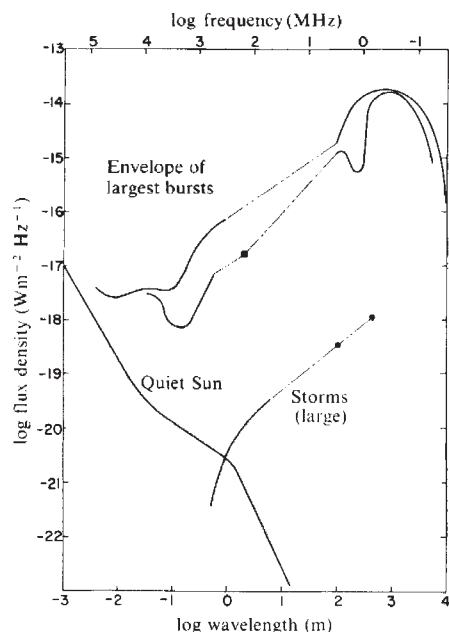


Fig. 1 Solar radio spectrum showing the envelope of flux levels reached by the largest reported bursts, one very large type III burst on 4 August 1972 (■), large noise storms, and the quiet Sun background. ●, RAE 1 measurements for a type III storm in August 1968.

approximately 3.2 times as many bursts with maximum flux density in the interval $S, S+\Delta S$ as in the interval $10S, 10(S+\Delta S)$.

Evans *et al.*¹¹ reported RAE 1 measurements at 107 and 430 m for a type III storm in August 1968; these data are indicated in Fig. 1. A wide variety of spectra for many individual type III bursts observed by IMP 6 have been presented elsewhere^{12,13}. Half of these bursts had spectral peaks occurring between 330 and 1,200 m; nearly all peaked within the 100 m–10 km observing range. The envelope of the largest long wavelength type III bursts, shown in Fig. 1, is derived from these IMP 6 data. This envelope should be considered as a lower limit for strongest bursts, because the IMP 6 observations cover only 500 days. The envelope of largest bursts at shorter wavelengths was assembled by Shimabukuro² from burst maxima observed between 1954 and 1972 by Badillo and Salcedo¹⁴ and Castelli *et al.*¹⁵. Taken together, the envelopes of largest bursts indicate that the maximum observed flux density continues to increase with increasing wavelength up to a spectral peak near 500 m.

Figure 1 also contains the spectrum for a particularly intense burst associated with a large proton flare on 4 August 1972. This burst was reported by Castelli *et al.*¹⁵ in the 3–60 cm range, by Solar Geophysical Data at 1.5 m and by me¹² in the 100 m–10 km range. Maximum flux density for the burst is observed between 330 and 1,500 m, wavelengths emitted at coronal levels of ~25–85 solar radii.

The quiet Sun spectrum is taken from Erickson *et al.*¹⁶, Aubier *et al.*¹⁷ and Linsky¹⁸. The spectrum for metric storms is from Wild *et al.*¹.

Solar radio burst flux densities have been observed far above quiet Sun flux levels, extending over seven decades of wavelength. These bursts are important indicators of solar activity. They are often associated with high-energy wave and particle emissions which influence the magnetosphere and upper atmosphere of the Earth. The long wavelength data contributed here show flux densities sometimes exceeding $10^{-14} \text{ W m}^{-2} \text{ Hz}^{-1}$ in large bursts, with the most intense radio burst emission observed at wavelengths near 500 m and generated at heliocentric distances of 20–65 solar radii.

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- Wild, J. P., Smerd, S. F. & Weiss, A. A. *Rev. Astr. Astrophys.* **1**, 291–366 (1963).
- Shimabukuro, F. I. in *The Solar Output and its Variation* (ed. White, O. R.) 133–150 (Colorado Associated University Press, 1977).
- Fainberg, J. & Stone, R. G. *Space Sci. Rev.* **16**, 145–188 (1974).
- Malitson, H. H., Fainberg, J. & Stone, R. G. *Astrophys. J. Lett.* **14**, 111–114 (1973).
- Malitson, H. H., Fainberg, J. & Stone, R. G. *Astrophys. J. Lett.* **183**, L35–L38 (1973).
- Brown, L. W. *Astrophys. J.* **180**, 359–370 (1973).
- Weber, R. R., Alexander, J. K. & Stone, R. G. *Radio Sci.* **5**, 1085–1097 (1971).
- Fitzenreiter, R. J., Fainberg, J. & Bundy, R. B. *Solar Phys.* **46**, 465–473 (1976).
- Steinberg, J.-L. & Caroubalos, C. *Astr. Astrophys.* **9**, 329–338 (1970).
- Akabane, K. *Publ. astr. Soc. Japan* **8**, 173–180 (1956).
- Evans, L. G., Fainberg, J. & Stone, R. G. *Solar Phys.* **21**, 198–203 (1971).
- Weber, R. R. NASA/GSFC X-692-78-12 (1978).
- Weber, R. R. *Solar Phys.* **59**, 377–385 (1978).
- Badillo, V. L. & Salcedo, J. E. *Nature* **224**, 503–504 (1969).
- Castelli, J. P., Barron, W. R. & Aarons, J. ARCL Rep. TR-73-0086 (1973).
- Erickson, W. C., Gergely, T. E., Kundu, M. R. & Mahoney, M. J. *Solar Phys.* **54**, 57–63 (1977).
- Aubier, M., Leblanc, Y. & Boischoit, A. *Astr. Astrophys.* **12**, 435–441 (1971).
- Linsky, J. L. *Solar Phys.* **28**, 409–418 (1973).

Chemical modification of a titanium (iv) oxide electrode to give stable dye sensitisation without a supersensitiser

SEMICONDUCTING electrodes stable in aqueous solution during the photoelectrochemical oxidation of water have generally been large-bandgap oxides, and their photosensitisation to visible light is of continuing interest¹. In particular, n-type TiO_2 (rutile, with a bandgap of 3.0 eV) is a stable photoanode for the photoelectrolysis of water by uv light², and considerable effort has been devoted to its photosensitisation to sunlight¹. One approach to photosensitisation is the use of a dye that, on excitation by visible light, transfers an electron to the conduction band of the solid and subsequently returns to its reduced ground state by the oxidation of water. The three major problems to be avoided are (1) irreversible degradative oxidation of the dye³, (2) failure of the oxidised form of the dye to oxidise water, reduction being carried out by a supersensitiser, such as hydroquinone, that is consumed by the reaction^{4,5}, and (3) inefficient

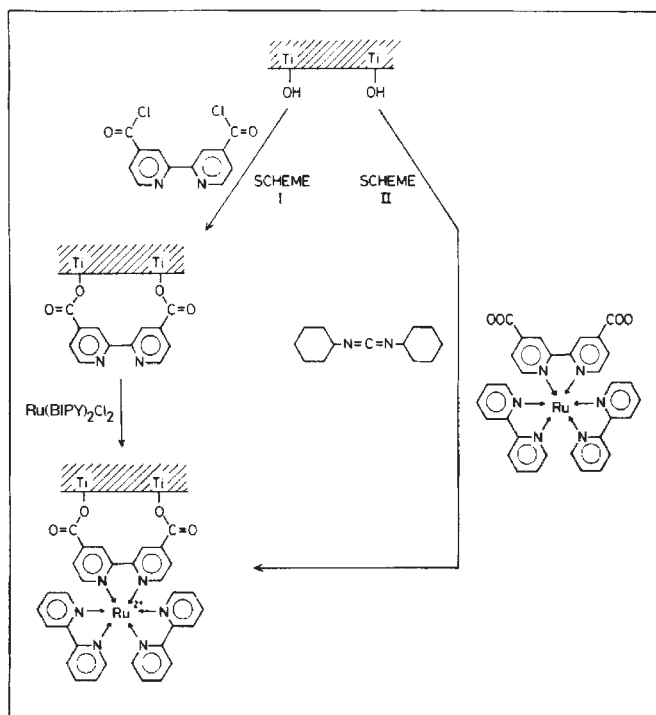


Fig. 1 Two reaction schemes for the derivatisation of TiO_2 with a monolayer of a $[\text{Ru}(\text{BIPY})_3]^{2+}$ derivative.

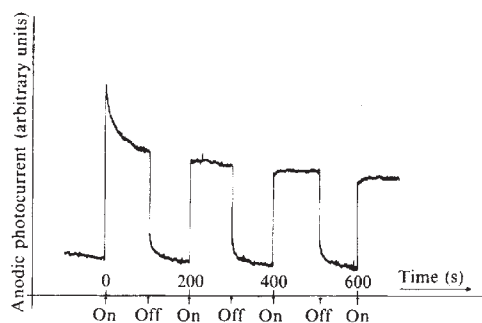


Fig. 2 Photocurrent-time characteristics for a derivatised TiO_2 electrode held at 1.2 V (versus SSE) and illuminated with light from a 150-W Xe lamp passing through a 450-nm interference filter. Arrows represent time of light switching on and off. Steady-state current was 70 nA cm^{-2} .

electron transfer from the dye to the solid⁶. To circumvent the first two of these problems, we have selected an inorganic complex as the dye, a derivative of $[\text{Ru}(\text{BIPY})_3]^{2+}$ (BIPY = 2,2'-bipyridine). $[\text{Ru}(\text{BIPY})_3]^{3+}$ is known to oxidise water in suitable pH conditions evolving about 80% of the theoretical yield of oxygen⁷. The last problem was avoided by chemically attaching the complex to the electrode surface, a procedure recently demonstrated for organic dyes (such as rhodamine-B) that require the use of a sensitizer^{4,5}. We report here that a single-crystal n-type TiO_2 electrode, chemically modified by the attachment of a monolayer of a derivative of $[\text{Ru}(\text{BIPY})_3]^{2+}$, will produce significant anodic photocurrents when irradiated with visible light in the absence of a sensitizer.

Two preparative routes (represented schematically in Fig. 1) were used to chemically modify single-crystal n-type TiO_2 electrodes. Both rely on the presence of electrode-surface sites that react like hydroxyl groups. Scheme I is a two-step process: derivatisation of the electrode surface with 2,2'-bipyridine-4,4'-di(carbonyl chloride), BPCC, followed by reaction with a solution of bis(2,2'-bipyridine)dichlororuthenium(II). Although BPCC has been prepared *in situ*⁸, we report⁹ its isolation and characterisation (by microanalysis, IR, NMR and mass spectrometry) for the first time. It was prepared by the reaction between 2,2'-bipyridine-4,4'-dicarboxylic acid, BPCA- H_2 , and thionyl chloride. Scheme II represents a one-step derivatisation reaction between bis(2,2'-bipyridine)(2,2'-bipyridine-4,4'-dicarboxylate)ruthenium(II), $[\text{Ru}(\text{BIPY})_2(\text{BPCA})]$, and dicyclohexylcarbodiimide (DCC). Electrodes modified by both these routes showed similar electrochemical behaviour, and we assume that the product in each case is a monolayer of a stable ruthenium complex attached to the electrode by an ester linkage.

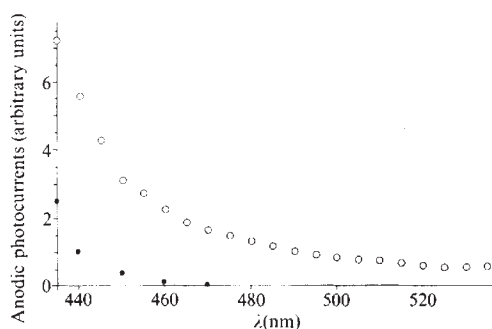


Fig. 3 Photocurrent-wavelength characteristics ('action spectrum') of a derivatised (○) and underderivatised (●) TiO_2 electrode held at 1.0 V (versus SSE) (corrected for the lamp profile).

The photoelectrochemical responses of both modified and unmodified electrodes were studied with a.c. and d.c. techniques capable of detecting very small currents¹⁰. Their response to light from a xenon lamp filtered to remove all radiation with $\lambda < 450 \text{ nm}$, a wavelength close to the absorption maximum of aqueous solutions of $[\text{Ru}(\text{BIPY})_3]^{2+}$ (ref. 11), was measured. As the absorption edge of TiO_2 is 420 nm, any observed photocurrent cannot be due to an electron excitation across the semiconductor bandgap. Indeed, neither the unmodified electrodes nor those modified only by the first step of Scheme I (Fig. 1) showed any significant photoresponse, whereas the fully modified electrode exhibited the response illustrated in Fig. 2, which clearly indicates the critical role of the ruthenium(II) moiety. The d.c. photocurrent is anodic and falls initially to a steady value; it does not exhibit the slow-rise-time cathodic component that has been observed¹⁰ with unmodified electrodes sensitised by $[\text{Ru}(\text{BIPY})_3]^{2+}$ in solution. The steady-state current may be maintained for many hours, with no obvious signs of diminution. From these observations we conclude that light absorbed by the complex excites electrons into the conduction band of TiO_2 , and the ruthenium(III) complex left on the surface is reduced back to ruthenium(II) by the capture of an electron from the solution. As no conventional sensitizer is present in the aqueous electrolyte (0.5 M sulphuric acid) in contact with the electrode, we thus demonstrate the direct catalytic photo-oxidation of water with visible light. The oxidation occurs at a rate faster than that observed for the chemical oxidation of water by $[\text{Ru}(\text{BIPY})_3]^{3+}$

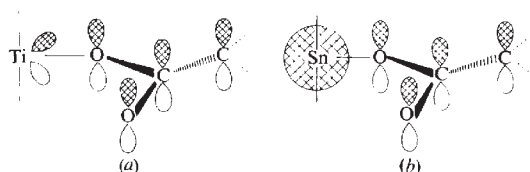


Fig. 4 Schematic representation of the orbitals of (a) esterified TiO_2 and (b) esterified SnO_2 . Crosshatching represents positive lobes, no crosshatching represents negative lobes.

in homogeneous solution¹², as might be anticipated for a redox couple in a charged monolayer. Moreover, the initial decrease in photocurrent may be attributed to an oxidation of surface states at energies above the ruthenium(III) level in addition to the oxidation of water. These would remain empty in the dark at the +1.0 V bias [versus standard silver electrode (SSE), which itself is +0.22 V versus standard hydrogen electrode] of measurement, and indeed the steady-state photocurrent returns immediately after a dark-cycle. On the other hand, the surface-states would be refilled if the bias voltage is cycled to -0.1 V during the dark period, and in fact such a programme recovers the initial photocurrent when the light is again switched on.

An intriguing feature of the photoresponse of the modified electrode is the absence of a peak near 450 nm in the action spectrum (illustrated in Fig. 3). Such a peak would be expected if electron transfer followed dye excitation, and peaks characteristic of dye absorption have been observed with isostructural SnO_2 electrodes modified by both chemically attached rhodamine-B^{4,5} and surfactant derivatives of $[\text{Ru}(\text{BIPY})_3]^{2+}$ deposited by a Langmuir-Blodgett technique^{12,13}. However, rhodamine-B attached to TiO_2 also gives a featureless action spectrum, similar to that shown in Fig. 3 (ref. 14), which suggests that, at TiO_2 , the dye electron is excited directly into the conduction band. In TiO_2 , the conduction band has a t_2 -orbital parentage, which may bond strongly with the π^* system of the ruthenium-bipyridine complex through the ester linkage (see Fig 4); the 5s conduction band of SnO_2 , on the other hand, is orthogonal to this π^* system. These observations suggest that the rate of electron transfer to the conduction band is much faster for TiO_2 than for SnO_2 modified electrodes. Experiments are in progress to further investigate and characterise these systems.

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1. Rajeshwar, K., Singh, P. & DuBow, J. *Electrochim. Acta* **23**, 1117–1144 (1978).
2. Harris, L. A. & Wilson, R. H. *A. Rev. Mater. Sci.* **8**, 99–134 (1978).
3. Maruska, H. P. & Ghosh, A. K. *Solar Energy* **20**, 443–458 (1978).
4. Osa, T. & Fujihira, M. *Nature* **264**, 349–350 (1976).
5. Fujihira, M., Ohnishi, N. & Osa, T. *Nature* **268**, 226–228 (1977).
6. Gerischer, H. & Willig, F. *Topics curr. Chemistry* **61**, 31–84 (1976).
7. Creutz, C. & Sutin, N. *Proc. natn. Acad. Sci. USA* **72**, 2858–2862 (1975).
8. Sprintschnik, G., Sprintschnik, H. W., Kirsch, P. P. & Whitten, D. G. *J. Am. chem. Soc.* **99**, 4947–4954 (1977).
9. Constable, E. C. & Seddon, K. R. (in preparation).
10. Hamnett, A., Dare-Edwards, M. P., Wright, R. D., Seddon, K. R. & Goodenough, J. B., *J. phys. Chem.* (in the press).
11. Demas, J. N. & Crosby, G. A. *J. Am. chem. Soc.* **93**, 2841–2847 (1971).
12. Memming, R. & Schröppel, F. *Chem. phys. Lett.* **62**, 207–210 (1979).
13. Memming, R. *Farad. Disc. Chem. Soc.* **58**, 261–270 (1974).
14. Wright, R. D. (in preparation).

Odd and even numbered hydrogen ion clusters

Since the suggestion that hydrogen ion clusters of the type H_n^+ may be present in gaseous nebulae^{1,2} and interstellar space^{3,4} and the early experiments of Clappitt and Gowland⁵, who discovered such clusters for $3 \leq n$ (odd) ≤ 99 in low temperature (3K) mass spectrometer experiments, it has sometimes been assumed that only odd-numbered clusters exist (ref. 6, and refs therein). There have been extensive studies in the past decade on the binding energy and the geometry of these ion-induced-dipole clusters. Massa *et al.*⁷ have carried out *ab initio* calculations on H_n^+ for odd values of n ranging from 3 to 15. Weak binding energies of the order of 44 meV against dissociation according to



were found. The geometrical shape of these clusters was calculated to be dominated by a triangular nucleating centre H_3^+ with the relatively high proton binding energy of 4 eV. Salmon and Poshusta⁸ have conducted polarised-orbital valence-bond calculations on the ground state properties of H_3^+ . Most recently, the molecular structure of H_3^+ has been experimentally determined by means of the beam-foil technique⁹. We describe here an ion-trap experiment in which we studied collisional processes of electrons with hydrogenic trapped ions of mass numbers, 1, 2, 3, 5, 7, 10, 14, and 20. Since these are the only ions observed, up to a mass limit of 40 AMU, there are serious implications for the assumption by some theoretical chemists that H_n^+ exists only for odd values of n .

The data were obtained in the hollow beam positive ion trap¹⁰ (nonspecific in mass) with concentric electron beam collision facility; this has previously been used for the study of ionisation of atomic ions by electrons¹¹ and the temperature dependence of dissociative recombination and electron-impact dissociation of molecular ions^{12,13}.

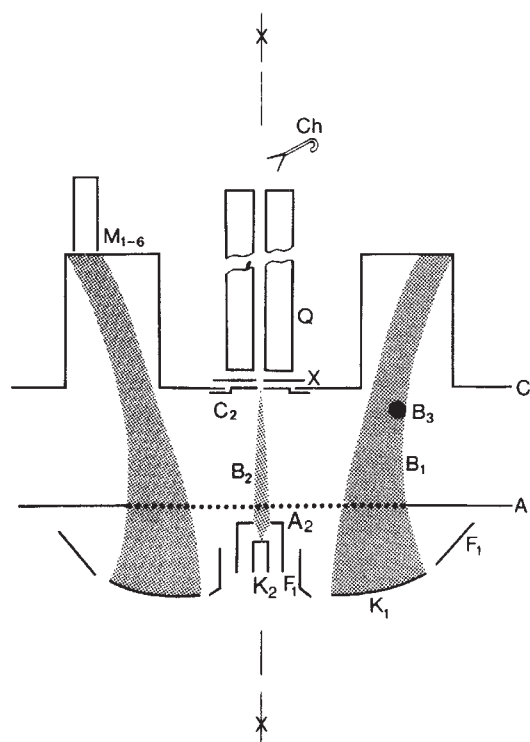


Fig. 1 Scale diagram of cylindrically symmetrical concentric electron beam device. Shaded beams: B₁, hollow electron beam; B₂, axial electron beam; B₃, molecular beam injected tangentially into hollow beam; K₁, toroidal cathode; K₂, rhenium strip filament; A₁, A₂, anodes; F₁, focusing electrode; C₁, hollow beam collector; C₂, axial beam collector; M₁₋₆, beam profile monitoring collectors; X, ion extraction electrode; Q, quadrupole mass spectrometer; Ch, channel electron multiplier.

The apparatus, shown in Fig. 1, consists of an indirectly-heated toroidal cathode which produces an intense electron beam; the potential configuration of the electron space charge produces a trap for positively-charged particles, the depth of which is of the order of 1 V. Advantage is taken of the capability of the hot nickel cathode to emit the species H_3^+ , from which other hydrogen cluster species are derived by electron-ion and ion-ion collisions. These processes dominate within the hollow beam, which is collisionally pumped to an ultrahigh vacuum ($\sim 10^{-8}$ torr). A molecular beam is injected at B₃ (Fig. 1) whilst the hollow electron beam B₁ is in operation. A quadrupole mass spectrometer sampling system detects the resulting ionic products.

The hollow beam is operated at a perveance of $15 \mu\text{A V}^{-3/2}$, with anode potentials V_{HB} , as shown in Fig. 2. It can safely be assumed that mass numbers 1, 2 and 3 correspond to the well-known H^+ , H_2^+ , and H_3^+ species. The definitive evidence of the assignment of mass 5 as H_3^+ is its dissociation¹³ by axial beam electron impact into $\text{H}_3^+ + \text{H}_2$ and also $\text{H}^+ + 2\text{H}_2$. An upper limit of 10^{-13} A can be placed on mass 4 ion current so that the well-known HeH^+ can be ruled out. Alternative assignments for the other mass numbers might be possible: HF^+ , 20; N^+ , 14; Li^+ , N^{2+} , 7; B^+ , 10; but each presents difficulties such as the absence of N_2^+ , 28; F^+ , 19; Li^+ , 6, or boron halide ions. In view of the absence of any other ion species in the trap (up to a mass limit of 40 AMU), the most likely conclusion is that all the ions in the trap are hydrogenic.

The intensities of ions of mass numbers 2, 3, 5, 7, 10, 14, and 20 are found to be independent of gas pressure over the range 10^{-8} to 10^{-5} torr, with slight increase in intensities in the region between 1×10^{-5} and 6×10^{-5} torr. The intensity of H^+ rises from 5×10^{-11} A at 2×10^{-7} torr to 1×10^{-7} A at 10^{-4} torr.

These cluster ions have been detected for the first time at laboratory temperatures because the hollow beam trap with surface-emitted H_3^+ ions successfully creates a situation in which

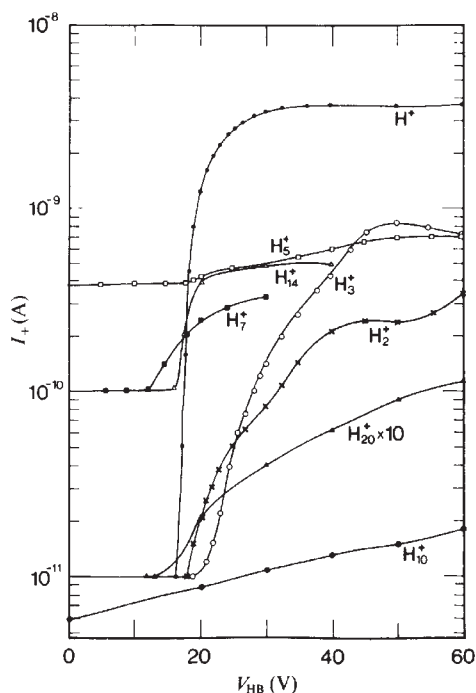
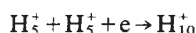
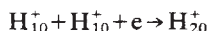


Fig. 2 Observed abundances of hydrogen ion clusters as functions of hollow beam energy V_{HB} .

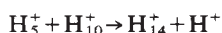
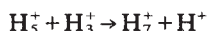
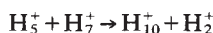
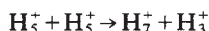
ion-neutral collisions, which would destroy clusters and complexes, contribute only marginally. It can be speculated that ion-ion collisions of two types are responsible for cluster formation in our device. First, there is the possibility of three-body collisions in which the third body is an electron:



H_5^+ is released from the nickel cathode surface, and is therefore independent of gas pressure and only marginally dependent on V_{HB} ; therefore, if this process dominates H_{10}^+ should exhibit similar behaviour. The data in Fig. 2 indicate that H_{10}^+ does indeed persist at the lowest hollow-beam anode voltage. Similar arguments can be applied to the reaction:



Second, it is possible that the observed ions are produced in the following types of process:



But why should odd-numbered clusters persist only up to $n = 7$, H_9^+ not being observed, although H_{10}^+ is stable? Conceivably in the low n odd-numbered clusters a proton orbits about H_2 molecules arranged orthogonally in one, two or three dimensions, so that a further extension of this principle beyond H_7^+ would not be possible.

No quantitative discussion of the cluster formation processes can be presented at this stage, but our data do indicate the need for theoretical chemists to initiate investigations of the hitherto unexplored problem of even-numbered H_n^+ clusters.

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1. Krasovskii, V. I. *Sov. Astr.* **2**, 715 (1958).
2. Donn, B. *Astrophys. J.* **132**, 507 (1960).
3. Hoyle, F., Wickramasinghe, N. C. & Reddish, V. C. *Nature* **218**, 112 (1968).
4. Wickramasinghe, N. C. & Reddish, V. C. *Nature* **218**, 661 (1968).
5. Clappitt, R. & Gowland, L. *Nature* **223**, 815 (1969).
6. Sapse, A. M., Rayez-Meame, M. T., Rayez, J. C. & Massa, L. J. *Nature* **278**, 332 (1979).
7. Harrison, S. W., Massa, L. J. & Solomon, P. *Nature phys. Sci.* **245**, 31 (1973).
8. Salmon, W. T. & Poshusta, R. D. *J. chem. Phys.* **59**, 4867 (1973).
9. Gaillard, M. J. *et al. Phys. Rev. A* **17**, 1797 (1978).
10. Hasted, J. B. & Awad, G. L. *J. Phys. B* **5**, 1719 (1972).
11. Hamdan, M., Birkinshaw, K. & Hasted, J. B. *J. Phys. B* **11**, 331 (1978).
12. Mathur, D., Khan, S. U. & Hasted, J. B. *J. Phys. B* **11**, 3615 (1978).
13. Mathur, D., Hasted, J. B. & Khan S. U. *J. Phys. B* **12**, 7921 (1979).

Processes controlling the stanol/stenol ratio in Black Sea seawater and sediments

STEROIDAL ALCOHOLS¹⁻¹⁰, ketones¹¹ and hydrocarbons^{12,13} have been used as tracers for the transformation processes of biogenic organic matter in Recent sediments. Several of these studies have been concerned with one of the sedimentary transformation reactions of steroidal alcohols, the reduction of stenols to stanols. However, little attention has been directed towards the role of the overlying water column in controlling sedimentary stanol/stanol distributions. The input of stanols from surface-water organisms coupled with relatively different degradation rates of stenols and stanols^{9,14} could also contribute to observed sediment distributions. To evaluate further the relative importance of these processes in controlling stanol/stanol distributions in anoxic water and surface sediments, we undertook a cruise to the Black Sea. Because water below 125 m is anoxic in the two central 2,000-m basins of the Black Sea, it is an ideal environment for observing any microbially or chemically mediated reduction reactions in the water column or surface sediments^{15,16}. In addition, since the surface sediments are anoxic, the effects of macrobenthos on these reactions are minimal. Indeed, sterol transformation reactions have been postulated to be microbially mediated in sediments^{1,2,7-9,11,13}, and in seawater¹⁷. However, very few, if any, bacteria are thought to biosynthesise desmethyl stenols^{17,18}. If stenol reduction reactions are chemically mediated, they should also be observed in this highly reducing (low E_H) environment. We discuss here the major processes controlling a specific transformation reaction (stenol to stanol conversion) of a class of biogenic organic compounds. These conclusions are based on results obtained by sampling both the sediments and the overlying water column from two stations in the Black Sea.

Seawater and sediment samples were collected on RV Chain Cruise 120, Leg 1 in April 1975. Core 7 was taken at hydrostation 1355, 42°50.0' N, 33°00.0' E, Core 23 was taken at 43°00.0' N, 34°00.0' E and hydrostation 1356 was taken at 42°41.3' N, 30°65.5' E. The procedure for collection of the water samples¹⁷ and cores¹⁹ and the interpretation of the hydrographic data¹⁷ are reported elsewhere. The presence of high concentrations of hydrogen sulphide as well as the absence of dissolved oxygen in the sediments and within the anoxic zone of the water column restrict life primarily to various species of microorganisms^{15,16}. Investigation of Recent sediments near Station 1355 has shown this environment to be of uniform deposition²⁰. The sediment analysed contained approximately 4 cm of a loose black floc above a grey unconsolidated organic-rich material. Karl¹⁶ found using scanning electron microscopy that the sediment contained coccoliths in various stages of dissolution and occasional diatom frustule fragments. In addition to these calcareous and siliceous detrital fragments, he observed many colonies of bacteria. A more detailed description of the sediment is published elsewhere¹⁹. The methods for the extraction, isolation, and quantification of the sterols are given in detail for seawater by R.B.G. and F.H.¹⁷, and for sediments by C.L. *et al.*^{5,6}. Structural assignments were based on high-resolution glass-capillary gas-chromatographic retention times of authentic sterol acetates in conjunction with glass capillary gas chromatography-mass spectrometry as described earlier^{6,17}.

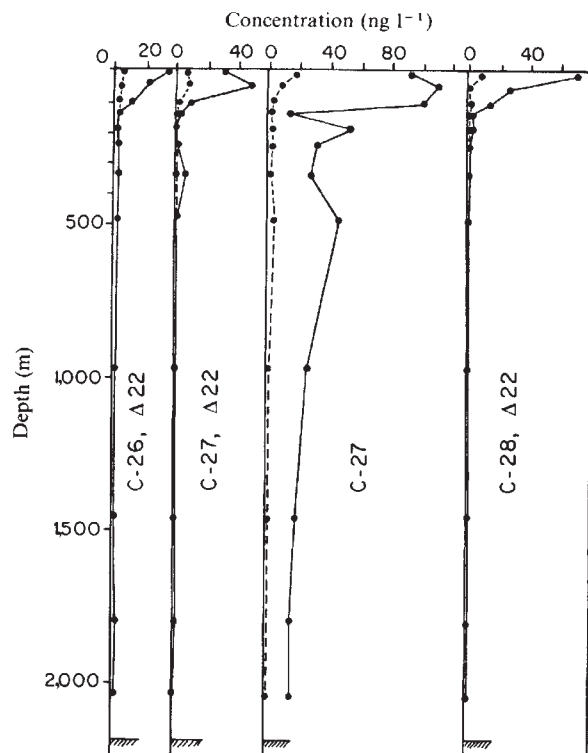


Fig. 1 Concentrations for four stanol (dashed lines), stanol (solid lines) pairs (I, II; III, IV; V, VI; VII, VIII) as a function of depth in the water column from Station 1355. See text for systematic names.

Sixteen stanols and stenols were resolved in the surface water samples from Stations 1355 and 1356 (ref. 17). Over 30 stenols were found in the surface sediments from Cores 7 and 23 (ref. 19). However, for the purpose of this report, we will limit our discussion to four clearly separable stanol/stenol pairs for both the seawater and sediment samples: 24-nor-5 α -cholest-22-en-3 β -ol(I)/24-norcholesta-5,22-dien-3 β -ol(II); 5 α -cholest-22(E)-en-3 β -ol(III)/cholesta-5,22(E)-dien-3 β -ol(IV); 5 α -cholestan-3 β -ol(V)/cholest-5-en-3 β -ol(VI); and 24-methyl-5 α -cholest-22-en-3 β -ol(VII)/24-methylcholesta-5,22-dien-3 β -ol(VIII). The concentrations of these stanol/stenol pairs as a function of depth in the water column for Station 1355 are shown in Fig. 1. Cholest-5-en-3 β -ol and 5 α -cholestan-3 β -ol are the only sterols detected above blank levels in waters deeper than the O₂/H₂S interface (located at 110–130 m). Similar results were found for two other stations in the Black Sea¹⁷. Although stanol Δ^5 bonds can be reduced chemically and microbiologically to both 5 α and 5 β stanols, only the 5 α isomer of cholestanol was detected. In the upper 100 m of the water column, all eight sterols were found in concentrations ranging from 5 to 93 ng l⁻¹. The surface water stanol/stenol ratios for compounds I–VIII are 0.15, 0.18 and 0.10 for the I/II, III/IV, V/VI, VII/VIII pairs respectively. These four sterol pairs were also found at the four depths sampled from Core 23 (Fig. 2) and the two depths sampled from Core 7. The 0–4-cm stanol/stenol ratios for the four respective pairs were found to be 0.28, 0.42, 1.17 and 0.45 for Core 23.

Since there is no evidence for sediment transport by turbidity currents at these locations and benthic sterol production is unlikely in the strongly anoxic water and sediments, the sedimentary sterols we observed were probably deposited by transport on large particles originating in the oxic zone but not captured by our seawater samplers. Recent studies using large-volume *in-situ* filtration systems²¹ and moored^{22,23} and free-floating²⁴ sediment traps indicate that the percentage of surface productivity rapidly reaching the sea floor directly below is much higher than previously thought. The large particles being transported potentially contain labile organic compounds since they reach the sea floor in a relatively short time (days).

The stanol/stenol ratio for the four corresponding pairs in the surface waters range from 0.10 to 0.18 (Fig. 1). These values are lower than those reported from surface sediments^{2,7,10}. They probably reflect the input from stanol-containing organisms (jellyfish²⁵, red algae²⁶, echinoderms²⁷ and sponge¹⁸) since the bacterial conversion of stenol to stanol would be expected to be slow in the oxic surface waters^{9,14}. From these comparatively high surface values, the concentrations of all the stanols decrease quickly with depth (faster than the corresponding stenols) to values near zero at the O₂/H₂S interface.

All of the stanol concentrations in the deep anoxic waters are below 5 ng l⁻¹. The only stanol/stenol pair that can be observed from the surface to the deep anoxic waters is the 5 α -cholestan-3 β -ol/cholest-5-en-3 β -ol pair (Fig. 3). The ratio drops from surface values of 0.15–0.20 to a ratio of about 0.05 below 100 m. The high value at 140 m depth from Station 1355 is due to an extremely low cholest-5-en-3 β -ol concentration rather than to an increase in 5 α -cholestan-3 β -ol (see Fig. 1). These results clearly show that no major chemical or microbial stanol $\rightarrow$ stanol conversion is occurring despite the highly favourable conditions at the interface and in the deep anoxic waters. The fact that the inflections of the stanol depth profiles are not mirrored by the corresponding stanol profiles¹⁷ (Fig. 1) also indicates very strongly that the stanol/stenol ratio in the oxic zone (upper 100 m) is primarily determined by varying inputs from specific source organisms.

Sediment ratios are quite different than those in the water column. In both Cores 7 and 23 the stanol/stenol ratio is 1.18, decreasing to about 1.0 with depth. Several processes may be responsible for this large difference between seawater and sediment ratios. First, as mentioned above, large particles probably transport sterols from the surface waters to the sediment surface. Stanol $\rightarrow$ stanol conversion could take place as these large particles transit to the sea floor. However, this conversion was not observed on the small particles collected by our water samplers. Since the small particles have a longer time for reaction due to their longer residence time in the water column, it is unlikely that this conversion occurs on the large particles. This reasoning assumes that the organic and microbiological compositions on the large particles are the same as on the small particles. This may not be true since the larger particles will have had much less time to degrade in the water column and may be a hormonal or more nutritional substrate. Plankton and other surface organisms are sources of the large particles. However, they usually do not contain more than 10% stanols relative to stenols^{8,9}. On the other hand, faecal pellets produced by these organisms are another source of large particulate matter^{21,22,24}, and may contain larger quantities of stanols formed by gut bacterial activity.

A second potential process for the increase in the stanol/stenol ratio in the surface sediments is selective degradation of stenols relative to stanols at the sediment/water interface. Enough stanols are produced in the surface waters to account for those we observed in the 0–4-cm sediment layer^{9,28} which represents about 500 years' accumulation^{19,20}. A selective degradation process is probably not occurring for two reasons.

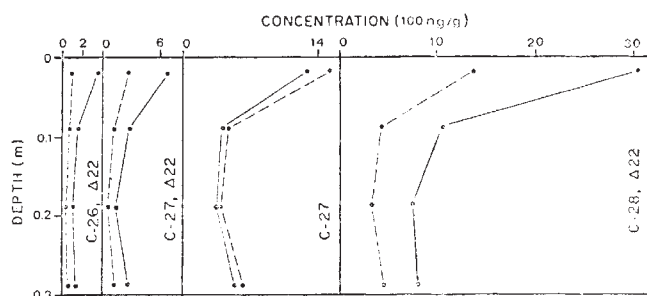


Fig. 2 Concentrations for four stanol (dashed lines), stanol (solid lines) pairs (I, II; III, IV; V, VI; VII, VIII) as a function of depth for Core 23. See text for systematic names.

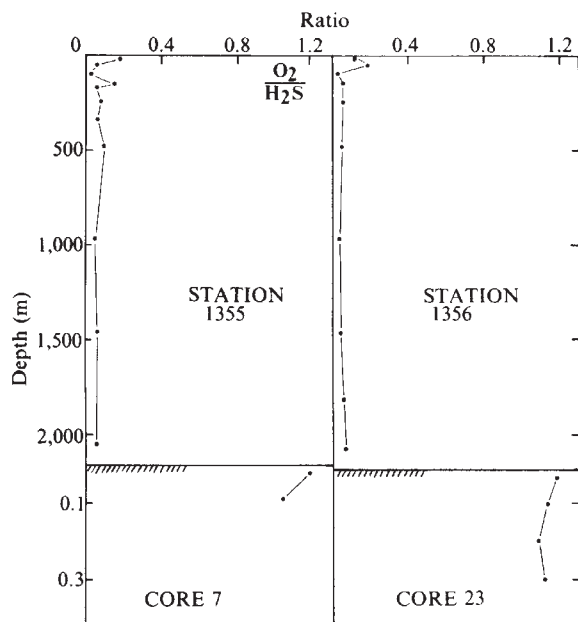


Fig. 3 Ratio of 5 α -cholestan-3 β -ol to cholest-5-en-3 β -ol as a function of depth in the water column from Stations 1355 and 1356 and in Cores 7 and 23.

First, although stenols degrade faster than stanols in oxic lacustrine sediments, and the stenol $\rightarrow$ stanol conversion is slow, the case in microbially active anoxic sediments is different. It seems that stenol degradation is suppressed and the reduction of stanols to stanols occurs rapidly, causing a marked increase in the stanol/stenol ratio with depth^{1,2,9,14}. Although this increase was observed in the surface sediments of the Black Sea, an increase with depth in the core was not found. However, the distribution of bacteria with depth and the sedimentation rate in the Black Sea can be expected to be quite different from those of lacustrine environments^{1,7,16}. Second, if stanols were selectively being degraded relative to stanols, we might have expected the 5 α - Δ 22 stanols to degrade faster than 5 α -cholestan-3 β -ol. This does not appear to be the case, as shown in Figs 1 and 2. 5 α -cholestan-3 β -ol(V) is over twice the concentration of 24-methyl-5 α -cholest-22-en-3 β -ol(VII) in the surface waters. However, the concentration of VII is about equal to V in the surface sediment. The same argument applies for the stability of I and III relative to V.

The third possible process controlling stanol/stenol ratios in the surface sediments is direct stenol $\rightarrow$ stanol conversion at the sediment/water interface. Since microbiological hydrogenation of stanols is well known²⁹⁻³¹, this pathway has been postulated as a major source of stanols in anoxic lacustrine sediments^{1,2,9,14}. The Black Sea sediments in this study have high microbial biomass and activity¹⁶. Hence, microbial transformation is the most likely control of stanol/stenol distributions in Black Sea surface sediments. However, input from large particles produced in the overlying water cannot be ruled out as a source of stanols. It is clear that in order to define the role large particles have in controlling surface sediment stanol/stenol distributions, sediment trap data is needed. We hope to collect such data in this most interesting anoxic environment in the near future.

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- Gaskell, S. J. & Eglinton, G. *Nature* **254**, 209-211 (1975).
- Gaskell, S. J. & Eglinton, G. *Geochim. cosmochim. Acta* **40**, 1221-1228 (1976).
- Huang, W. Y. & Meinschein, W. G. *Geochim. cosmochim. Acta* **40**, 323-330 (1976).
- Ikan, R., Baedecker, M. J. & Kaplan, I. R. *Geochim. cosmochim. Acta* **39**, 195-203 (1975).
- Lee, C., Gagosian, R. B. & Farrington, J. W. *Geochim. cosmochim. Acta* **41**, 985-992 (1977).
- Lee, C., Farrington, J. W. & Gagosian, R. B. *Geochim. cosmochim. Acta* **43**, 35-46 (1979).
- Nishimura, M. *Geochim. cosmochim. Acta* **41**, 1817-1823 (1977).
- Nishimura, M. & Koyama, T. *Chem. Geol.* **17**, 229-239 (1976).
- Nishimura, M. & Koyama, T. *Geochim. cosmochim. Acta* **41**, 379-385 (1977).
- Wardrop, A. M. K., Maxwell, J. R. & Morris, R. J. *Steroids* **32**, 203-221 (1978).
- Gagosian, R. B. & Smith, S. O. *Nature* **277**, 287-289 (1979).
- Dastillung, M. & Albrecht, P. *Nature* **269**, 678-679 (1977).
- Gagosian, R. B. & Farrington, J. W. *Geochim. cosmochim. Acta* **47**, 1091-1101 (1978).
- Nishimura, M. *Nature* **270**, 711-712 (1977).
- Jannasch, H. W., Truper, H. G. & Tuttle, J. H. in *The Black Sea—Geology, Chemistry and Biology* (eds Degens, E. T. & Ross, D. A.) 419-425 (AAPG, Tulsa, 1974).
- Karl, D. M. *Limnol. Oceanogr.* **23**, 936-949 (1978).
- Gagosian, R. B. & Heinzer, F. *Geochim. cosmochim. Acta* **43**, 471-486 (1979).
- Nes, W. R. & McKean, M. L. *Biochemistry of Steroids and Other Isoprenoids*, 411-517 (University Park Press, Baltimore, 1977).
- Lee, C., Gagosian, R. B. & Farrington, J. W. *Org. Geochem.* (in the press).
- Ross, D. A., Degens, E. T. & MacIvaine, J. *Science* **170**, 163-165 (1970).
- Bishop, J. K., Edmond, J. M., Ketten, D. R., Bacon, M. P. & Silker, W. B. *Deep Sea Res.* **24**, 511-548 (1977).
- Honjo, S. *J. mar. Res.* **36**, 469-492 (1978).
- Spencer, D. W. *J. mar. Res.* **36**, 493-523 (1978).
- Staresinic, N. thesis, WHOI/MIT Joint Program in Biological Oceanography (1978).
- Ballantine, J. A., Roberts, J. C. & Morris, R. J. *Biomed. Mass Spect.* **3**, 14-20 (1976).
- Chardon-Lorrain, L., Morisaki, M. & Ikegawa, N. *Phytochemistry* **15**, 723-725 (1976).
- Goad, L. J. in *Biochemical and Biophysical Perspectives in Marine Biology* Vol. 3 (eds Malins, D. C. & Sargent, J. R.) 213-318 (Academic, London, 1972).
- Gagosian, R. B. & Nigrelli, G. N. *Limnol. Oceanogr.* (in the press).
- Eyssen, H. J., Parmentier, G. C., Compennoll, F. C., DePauw, G. & Piessens-Denet, M. *Eur. J. Biochem.* **36**, 411-421 (1973).
- Rosenfeld, R. S. & Hellman, L. J. *Lipid Res.* **12**, 192-197 (1971).
- Schubert, K. & Kaufmann, G. *Biochim. biophys. Acta* **106**, 592-597 (1965).

Analysis of aliphatic and aromatic hydrocarbons in Antarctic marine sediment layers

THE presence of both paraffinic and polycyclic aromatic hydrocarbons (PAH) in sediments and soils from the Northern Hemisphere has been well documented¹⁻⁵. Paraffinic hydrocarbons are generally assumed to be derived predominantly from land plants⁶ but the sources of PAH are still uncertain⁷. However, the general consensus seems to be that they are from the fallout of airborne combustion products³. Industrialised areas have higher PAH loadings⁵ whilst dated cores from areas with a history of increasing industrialisation show a concomitant increase in total PAH loads but no great change in the relative proportions of individual components. Considerable interest is also vested in the fate of hydrocarbons once deposited in sediments⁶. With mineral resources being depleted in more accessible areas, those of remote regions (including polar ones) are being considered. But little is yet known of the effects that near-zero temperatures may have on the rate of biochemical or chemical alteration of hydrocarbons such as those found in petroleum. We report here our attempts to test the 'combustion source hypothesis' and to investigate the fate of hydrocarbons in sediments from a remote sub-Antarctic island with a well-documented history of localised industrial pollution⁸ spanning the period 1904-65. Only very minor local inputs of pollutant hydrocarbons are likely to have occurred on either side of these dates.

South Georgia (54°S, 37°W) became an important seal hunting ground during the first half of the nineteenth century. But with the virtual extinction of the local seals it was then little used until 1904, when a whale processing factory was built at King Edward Cove. Thereafter, fossil fuels (first coal and then, increasingly from about 1925, fuel oil) were transported to the factory to fire cookers and catchers. Fossil fuel contamination of the bay occurred especially when the tanks of the transporters were cleaned before reloading with whale oil. In addition, large quantities of organic material entered the bay as a result of

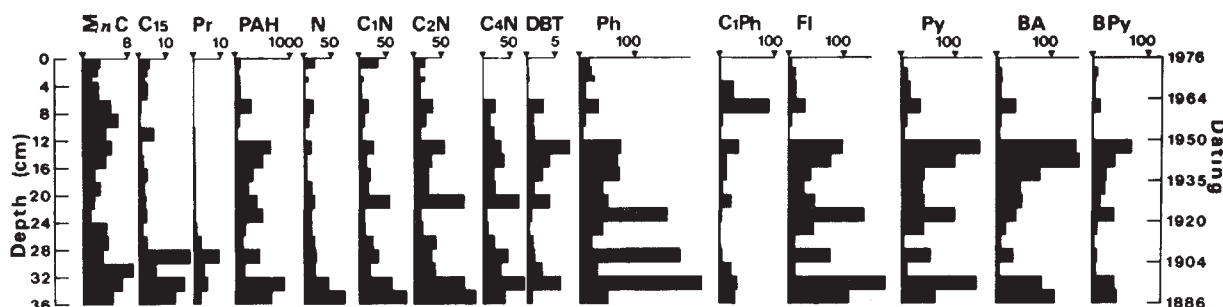


Fig. 1 Depth distribution of selected hydrocarbons in a marine sediment core from South Georgia (units are ng per g dry wt except for *n*-C and Pr ($\mu\text{g per g}$) and C_{15} (% of $\Sigma n\text{-C}$)). For abbreviations see Table 1.

processing the whales, most markedly before 1916 when only the blubber was utilised. The factory closed in 1965 and the site subsequently returned to light use, with a small scientific station being visited by occasional research and fishing vessels.

In 1976, divers collected cores from 18 m in the deepest part of the bay. Sections of a 2.2-cm diameter, 36-cm long core were extracted⁹ and the alkane and PAH fractions isolated according to the method of Giger and Schaffner¹⁰. The quantitative alkane data were obtained by glass capillary GC¹¹. Analyses of the PAHs were also performed on a glass capillary column but used a mass spectrometer, operated in the multiple ion mode, as a detector¹¹. Identifications are based primarily on retention indices with random peaks scanned to confirm identities. A minimum of two injections of a standard mixture were made daily to confirm both retention time and spectrometer response. The parent ion was used for quantitation on all components except for the C1 and C2 substituted naphthalenes for which the M-1 and M-15 ions respectively were used.

The sediment core results are shown in Fig. 1 and the South Georgia whale oil (grade 2) and fuel oil (from abandoned storage tanks) data are given in Table 1. Figure 1 also shows a putative dating based on inferences made from the hydrocarbon and organic carbon profiles of the cores and from sedimentation experiments¹². The estimate of the 1975–76 annual sedimentation rate based on sediment trap data agreed well with the implications of assuming that the unusual carbon profile reflected the increased organic input to the sediment during the whaling years. As this input of waste may have altered the sediment accumulation rate it is not easy to state the reliability of the precise dating between 1904 and 1965. However, all the available evidence seems to be consistent with the conclusion that the sediment at 30 cm depth (in 1976) was laid down in 1904 and that the average annual sedimentation rate thereafter was of the order of $2.8 \times 10^3 \text{ g dry wt m}^{-2}$.

Present evidence suggests bioturbation could effectively backdate any dating by about 10 yr (any material laid down in 1 yr would be mixed with sediment laid down up to 10 yr previously). The macrobenthic fauna in the area¹³ consists predominantly of burrowing polychaetes in the upper 2–4 cm aerobic layers. However, organic inputs from the whaling operations may have either altered the benthic fauna so as to increase bioturbation or reduce it and so decrease such mixing. Evidence from other areas subjected to massive organic pollution suggest the macrofauna would have been eliminated^{14–15} so a large dating error due to macrobenthic activity between 1904 and 1965 is unlikely. Nevertheless, it is still possible that a persistent meiofaunal population could have survived to cause some mixing¹⁶.

The upper sections of the core can be considered free of local pollution: most of the aliphatic components are entirely consistent with their being locally derived, from marine organisms and land plants¹⁷. However, as biogenic PAH synthesis is considered unlikely¹⁸, the aromatic components would seem to be the result of a world-wide dissemination of natural or anthropogenic combustion effluents and/or petroleum. The low ratio of parent-to-alkylated phenanthrenes effectively rules out petroleum as a

source, petroleum being low in unsubstituted species. This leaves combustion products from continental land masses as the prime source, as evidenced by the roughly equal proportions of fluoranthene to pyrene and the predominance of parent tricyclic over alkylated tricyclics³. Thus, the results from South Georgia support the hypothesis that sedimentary PAH are combustion derived.

The mean PAH concentration in the top 3.5 cm of the King Edward Cove mud (100 parts per 10^9 (p.p.b.) dry wt) was only eight times less than that found in the fine sands of Buzzards Bay⁷, a shallow (17 m) site near the heavily industrialised northeastern seaboard of the US and just slightly less than the 160 p.p.b. figure for the (presumably) finer grained surficial sediments at 130 m depth 94 km west of Boston (Massachusetts)⁴. Therefore, despite the fact that PAH concentration is a function of sediment type as well as relative input, the concentrations found at South Georgia still seem to be surprisingly high for such an isolated location, indicating that global contamination reaches even remote parts of the world in relatively undiminished quantities.

The apparent presence of small amounts of PAH in the whale oil deserves comment. Table 1 shows that the relative proportions of the component PAHs bear a similarity to those of the fuel oil so that it seems that the whale oil probably became contaminated by petroleum at some stage.

Anthropogenic combustion of fossil fuels has been continuously increasing during this century⁷, so that the greater hydrocarbon concentrations deeper in this core could only have resulted from the local industry. Indeed, the abrupt increase in many components at the 6–8 cm depth seems to coincide with the cessation of industrial activity. The obvious discontinuities in both amounts and distribution must reflect local contamination

Table 1 Hydrocarbon composition of whale oil and fuel oil from South Georgia

Component	Whale oil	Fuel oil
Total <i>n</i> -alkanes $C_{15}\text{--}C_{33}$ ($\Sigma n\text{-C}$) ($\mu\text{g per g}$)	8.9	4.9×10^4
Normal pentadecane (C_{15}) (% of $\Sigma n\text{-C}$)	59.6	8.1
Pristane (Pr) ($\mu\text{g per g}$)	322.7	1550.9
Total identified PAHs (ng per g)	86.2	3.3×10^6
Naphthalene (N) (ng per g)	12.4	0.2×10^6
Methylnaphthalene (C_{1N}) (ng per g)	14.3	0.7×10^6
Dimethylnaphthalene (C_{2N}) (ng per g)	17.0	2.2×10^6
Tetramethylnaphthalene (C_{4N}) (ng per g)	ND	0.4×10^6
Dibenzthiophene (DBT) (ng per g)	2.0	9.6×10^4
Phenanthrene (Ph) (ng per g)	5.8	5.5×10^4
Methylphenanthrene/anthracene (C_{1Ph}) (ng per g)	26.1	10.8×10^4
Fluoranthene (F1) (ng per g)	0.6	ND
Pyrene (Py) (ng per g)	1.8	0.5×10^4
Benzantracene/phenanthrene (BA) (ng per g)	6.6	3.6×10^4
Benzo(a)pyrene (BPy) (ng per g)	1.6	0.4×10^4

ND, not detected.

as all other sources should in principle give relatively homogeneous distributions. These discontinuities further imply that neither diffusion nor sediment reworking was a major factor during these years. In general, the qualitative distribution of the PAH below 8 cm is again consistent with combustion being the source, with inputs from the whaling station far exceeding allochthonous inputs.

The total *n*-alkanes are most abundant in the 6–14 cm and 30–36 cm regions. The lower peak is a legacy of the wasteful early years of whaling, as illustrated by the *n*-pentadecane and pristane data (both major constituents of whale oil). The upper alkane peak coincides with increasing post-1945 use of fuel oil, indicated by reduced carbon preference index (CPI) values¹⁹ and enhanced phytane concentrations: CPI of 1.15 in 8–12 cm section compared with a normal value of 1.65 and phytane concentration of 0.3 µg per g in 10–14 cm section compared with a normal value of 0.1 µg per g.

However, the various indicators of the presence of petroleum are scattered but not coincidental throughout the sections dated 1945–69 (3.5–14 cm). Thus, if the phytane values¹⁹ are examined, the greatest concentration of petroleum is from 1945 to 1955, if the ratio of parent to substituted tricyclics²⁰ is used, oil is present mainly in the 1959–69 regions whilst the amount and distribution of alkanes¹⁹ suggest that 1955–60 are the years of major fuel oil input. No reasonable explanation is evident for this phenomenon, which must await a closer understanding of the fate of petroleum hydrocarbons in cold benthic ecosystems. Clearly these results represent evidence for the long-term stabilisation of petroleum components in these conditions, at least 20 yr—apparently the longest so far reported.

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- Grimmer, G. & Böhnke, H. *Cancer Lett.* **1**, 75 (1975).
- Hites, R. A. & Biemann, W. G. in *Analytical Methods in Oceanography* (ed. Gibb, T. R. O.) *Adv. Chem. Ser.* **147** (Washington, D.C. 1975).
- Laflamme, R. E. & Hites, R. A. *Geochim. cosmochim. Acta* **42**, 289 (1978).
- Windsor, J. G. & Hites, R. A. *Geochim. cosmochim. Acta* **43**, 27 (1979).
- Youngblood, W. W. & Blumer, M. *Geochim. cosmochim. Acta* **39**, 1303 (1975).
- Farrington, J. W. & Tripp, B. W. *Geochim. cosmochim. Acta* **41**, 1627 (1977).
- Hites, R. A., Laflamme, R. E. & Farrington, J. W. *Science* **198**, 829 (1977).
- Platt, H. M. *Mar. Pollut. Bull.* **9**, 149 (1978).
- Mackie, P. R., Whittle, K. J. & Hardy, R. *Estuar. coast. mar. Sci.* **2**, 359 (1974).
- Giger, W. & Schaffner, C. *Analyt. Chem.* **50**, 243 (1978).
- Mackie, P. R., Hardy, R. & Whittle, K. J. *J. Fish. Res. Board Can.* **35**, 544 (1978).
- Platt, H. M. *Estuar. coast. mar. Sci.* (in the press).
- Platt, H. M. *Bull. Br. Antarct. Surv.* **51** (in the press).
- Rosenberg, R. *Oikos* **27**, 414 (1976).
- Christie, N. D. & Moldan, A. *Mar. Pollut. Bull.* **8**, 41 (1977).
- Cullen, D. J. *Nature* **242**, 323 (1973).
- Mackie, P. R., Platt, H. M. & Hardy, R. *Estuar. coast. mar. Sci.* **6**, 301 (1978).
- Hase, A. & Hites, R. A. *Geochim. cosmochim. Acta* **40**, 1141 (1976).
- Keizer, P. D., Dale, J. & Gordon, D. C. *Geochim. cosmochim. Acta* **42**, 165 (1978).
- Teal, J. M., Burns, K. & Farrington, J. J. *J. Fish. Res. Board Can.* **35**, 510 (1978).

Ordovician conglomerates and the evolution of the Midland Valley

ORDOVICIAN and Silurian marine clastic sequences containing boulder bearing conglomerates overlie the Ballantrae ophiolite complex in South-west Ayrshire, Scotland. These conglomerates are thought to have been emplaced by submarine slides¹ or less viscous marine currents². The Ordovician conglomerates accumulated at the toes of contemporary submarine faults downthrowing to the south³ and in both their stratigraphy and palaeocurrents there is substantial evidence for

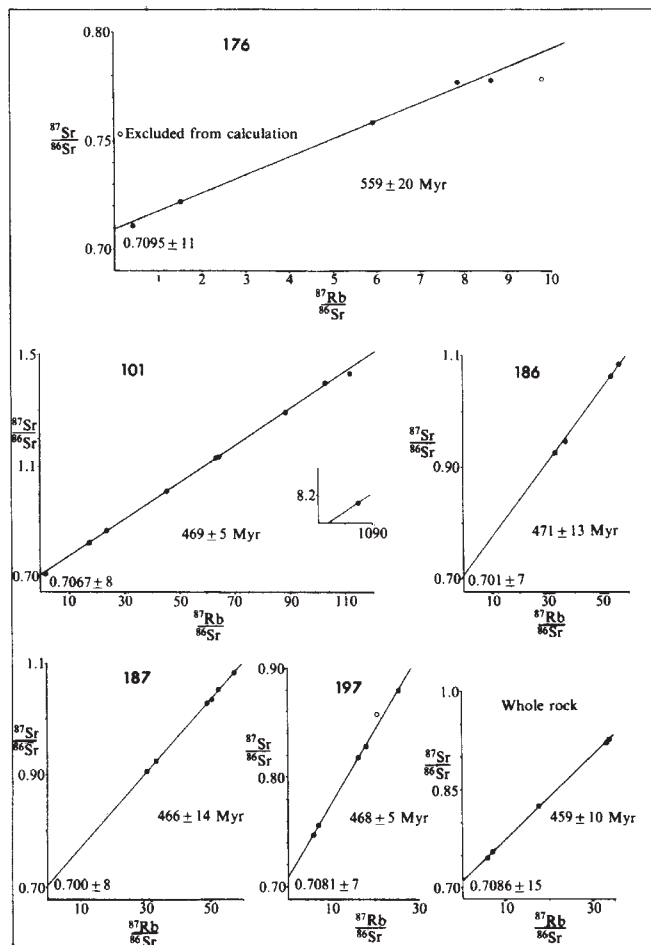


Fig. 1 Mineral isochrons of five different granite clasts and one composite whole rock isochron.

a northerly source. The conglomerates contain clasts of the underlying ophiolite, metamorphic rocks, acid and intermediate volcanic and hypabyssal rocks and clasts of undeformed pink granite some of which are >80 cm in largest dimension. Dewey⁴ and many subsequent workers⁵ envisage these sediments as having accumulated on the southern margin of a continent under which oceanic plate was being consumed in a northerly dipping subduction zone. The time span of this subduction is subject to much speculation⁶ but is generally thought to have ceased by Devonian time. We report here our investigations of the clasts in the southern Ayrshire conglomerates to elucidate (1) the nature and extent of plutonic activity in the area to the north of the subduction zone, and (2) the nature of the crust into which the plutons were emplaced.

Rb–Sr isotope dilution analyses of these clasts follow the analytical methods described by Blaxland *et al.*⁷. Rb–Sr ages have been calculated, or recalculated, using $\lambda^{87}\text{Rb} = 1.42 \times 10^{-11} \text{ yr}^{-1}$. Analytical uncertainties at the 1σ level for $^{87}\text{Rb}/^{86}\text{Sr}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios are $\pm 0.7\%$ and $\pm 0.03\%$ respectively. Seven analyses of the NBS 607 potassium feldspar standard yield average $^{87}\text{Rb}/^{86}\text{Sr}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ values of 24.21 ± 11 (1σ) and 1.2006 ± 6 (1σ) respectively. The average $^{87}\text{Sr}/^{86}\text{Sr}$ value for NBS 987 standard SrCO_3 is 0.71020 ± 5 (1σ). Rb–Sr isochron ages were calculated using the least squares method of York⁸. All isochrons are concordant (MSWD < 2.5) or near concordant suggesting the observed scatter to be analytical rather than geological.

Rb–Sr analytical data are shown in Table 1. Mineral isochrons determined for five different granite clasts (minimum of 30 cm in longest dimension) and one composite whole rock isochron are plotted in Fig. 1.

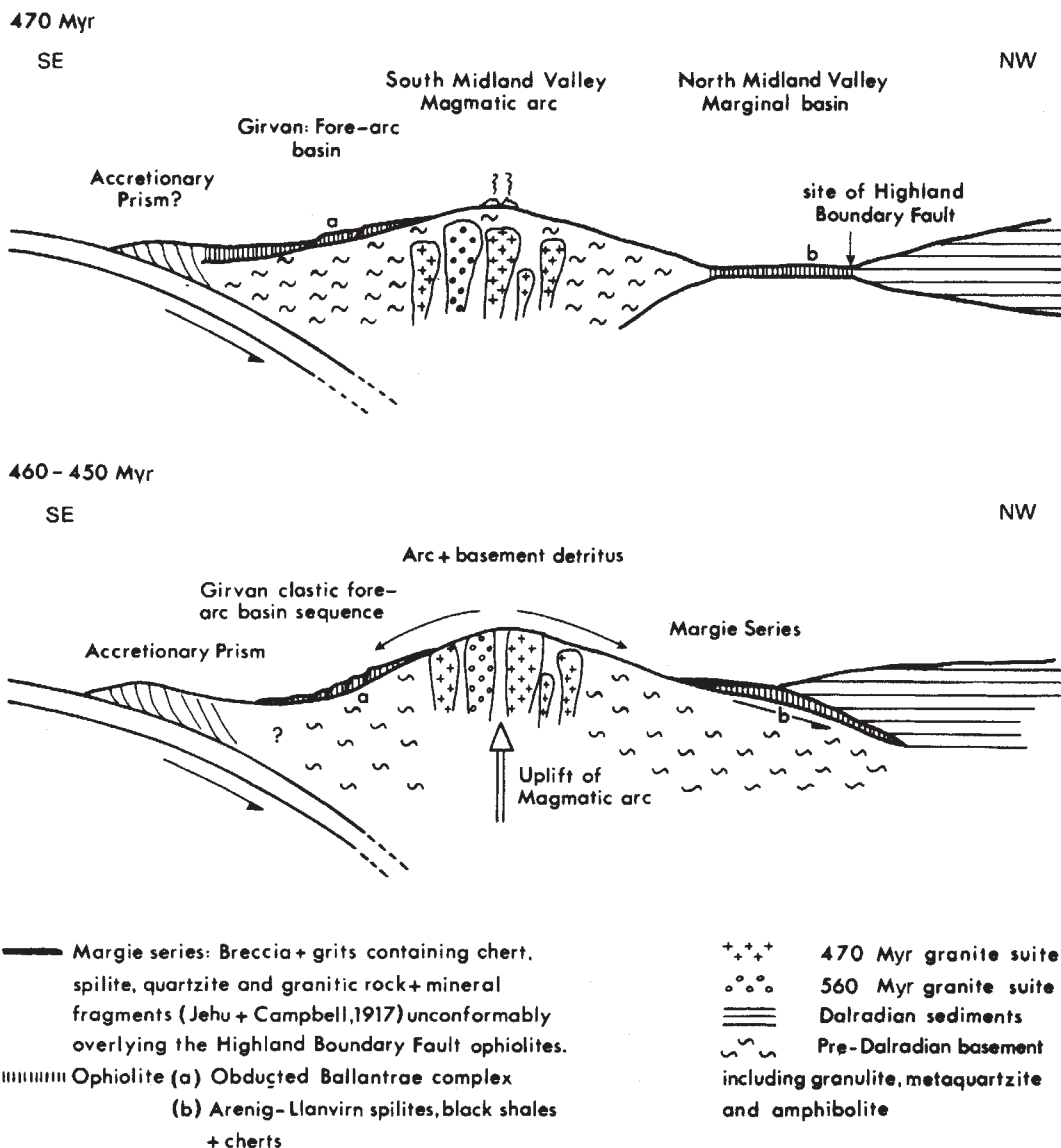


Fig. 2 Reconstruction of the Midland Valley during the Ordovician.

The age values obtained suggest a two-fold division defining one event at ~560 Myr and another at ~470 Myr. Sample 176, an undeformed non-perthitic biotite-chlorite bearing subsolvus granodiorite⁹ yields an age of 559 ± 20 Myr. The other four clasts, 101, 196, 187 and 197 yield ages of 469 ± 5 , 471 ± 13 , 466 ± 14 and 468 ± 5 Myr respectively. These samples are less mafic, commonly micropertthite-bearing subsolvus granites. The four whole rock points from the samples defining the ~470 Myr event were plotted along with another whole rock point from a chemically and petrographically similar clast (Fig. 1). These define a tentative isochron of 459 ± 10 Myr.

The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of three younger samples agree within experimental error (those for 186 and 187 were excluded as being ill defined and anomalously low) ranging from 0.7067 ± 8 to 0.7087 ± 15 and giving a mean of 0.7078 ± 10 . The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio for sample 176 is 0.7095 ± 11 .

The age and stratigraphic position of the granite clasts places constraints on the Ordovician time scale. The dated clasts come from the Benan conglomerates of Llandeilo age. Petrographically similar clasts are, however, found in conglomerates ranging through to Llandovey in age¹⁰. Presently observed rates of uplift and erosion¹¹ suggest that surface exposure of the granites is possible in <10 Myr. This gives a maximum age for the base of the Llandeilo of about 460 Myr. This figure is in reasonable agreement with some published Ordovician time scales^{12,13} based largely on K-Ar and Rb-Sr ages, but is irreconcilable with older ages based on fission track data¹⁴.

Consideration of dispersal of the conglomerates and the size of the granite clasts indicate a provenance to the immediate north, in the south-west margin of the Midland Valley. The granite clasts have a similar petrography and age to the exposed pre and late tectonic granites in the Highlands. Ages of ~450 Myr are reported for many of the smaller granites of North-east Scotland¹⁵ whereas the Carn Chuinneg-Inchbae granite is dated at 560 ± 10 Myr^{16,17}. The known pre and late tectonic plutonic activity which preceded the ubiquitous ~400 Myr granites, relating to the final closure of the Iapetus Ocean, is therefore extended south of the Highlands.

The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of the Carn Chuinneg granite (0.710 ± 2)¹⁶ is similar to that of sample 176—unless the 559 ± 20 Myr age for this rock is a cooling age and the high $^{87}\text{Sr}/^{86}\text{Sr}$ ratio reflects Sr isotopic equilibration on a mineral scale. In contrast, the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of around 0.708 for the ~470 Myr suite are significantly lower than the values of 0.714–0.717 for the ~450 Myr suite of Aberdeenshire granites¹⁵. Taken together, the 470–450 Myr Midland Valley–Aberdeenshire granites show a northerly increase in initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. This pattern together with the high normative corundum and presence of muscovites in the northern granites¹⁸ can be explained by increasing crustal involvement in the northern source. Although this evidence does not prove northward subduction the data are consistent with $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio patterns of Mesozoic and Cenozoic subduction related granites in the western US^{19,20} and such a model accords with

Table 1 Analytical data

Sample	Phase	Rb (p.p.m.)	Sr (p.p.m.)	⁸⁷ Rb/ ⁸⁶ Sr	⁸⁷ Sr/ ⁸⁶ Sr
17600	Whole rock	94.9	177.8	1.5640	0.72212
17607	Plagioclase	57.8	374.8	0.4465	0.71274
17613	Alkali feldspar	264.0	130.0	5.9044	0.75801
17616		287.7	106.9	7.8362	0.77133
17617		296.1	100.1	8.6205	0.77687
17618		312.3	92.5	9.8451	0.78417
10100	Whole rock	163.8	27.1	17.6832	0.82536
10107	Perthite	35.2	86.0	1.1833	0.71460
10113		450.6	21.5	63.1951	1.13128
10115		591.6	17.7	103.374	1.39660
10116	Muscovite	1049.1	4.8	1084.75	8.17221
10104*	Perthite	284.3	35.0	23.8428	0.86476
10108*		389.5	25.6	45.3412	1.00714
10111*		445.5	21.0	64.1603	1.13346
10113*		521.6	18.1	88.2863	1.29343
10114*		607.6	16.9	111.411	1.43777
18600	Whole rock	112.0	10.5	33.8337	0.92777
18605	Perthite	173.3	14.0	36.7596	0.94866
18612		244.5	13.6	53.9222	1.06083
18613		258.9	13.7	56.7424	1.08367
18700	Whole rock	127.1	11.1	33.9507	0.92320
18705	Perthite	142.4	13.7	30.6406	0.90431
18709		227.1	13.7	49.2947	1.02998
18711		226.9	13.4	50.5927	1.03354
18713		235.9	13.4	52.6500	1.05118
18714		258.0	13.4	57.8238	1.08135
19700	Whole rock	117.2	48.1	7.0899	0.75569
19704	Alkali feldspar	133.2	65.4	5.9144	0.74731
19706		256.6	45.8	16.4009	0.81789
19708		280.2	45.4	18.0603	0.82799
19709		306.0	43.5	20.6541	0.85765
19710		364.9	41.7	25.7471	0.87975
19400	Whole rock	117.4	61.2	5.5704	0.74441

All mineral separates are of a size fraction 210–175 μm except those marked * which are 500–210 μm .

many views on plate configuration in this region at that time²¹.

The discovery of granite boulders of this age, together with consideration of the type and distribution of clasts in other Midland Valley conglomerates helps to refine existing models. Boulders and large cobbles of granite, quartz-porphry, rhyolite and andesite present in Ordovician and Silurian conglomerates, suggest major intrusive and extrusive activity in the source area. The plutonic activity is now dated, and Kelling²² in recording an influx of andesite fragments in Llandeilo greywackes has suggested volcanic fields in this region at the same general time as the intrusion of the younger granites of this study. This evidence is consistent with the development of an island arc in the southern margin of the Midland Valley reaching maturity in Lower Ordovician time. The existence of an island arc associated with the northward subduction has been previously postulated^{4,23,24} but hitherto no evidence for its existence has been available.

Cobbles and boulders of metaquartzite, amphibolite and sheared granite occur in the northerly derived Ordovician conglomerates discussed here and the southerly derived Silurian conglomerates of the southern Midland Valley²⁵. Metaquartzite thus formed part of the provenance in the Midland Valley at this time. However, as the 560 Myr granite is unfoliated it seems likely that the event which created the metamorphic assemblage pre-dated the early igneous episode. Furthermore, the presence of metaquartzite suggests ensialic deposition. Thus the evidence points to the existence of Precambrian basement under the Midland Valley, in accord with the original interpretation placed on granulite clasts of intermediate composition found in Carboniferous vents²⁶. Mineral chemistry of these clasts indicate pressures and temperatures of metamorphism exceeding 11 kbar and 850 °C (ref. 27) and recent Sm–Nd isotopic work indicates a maximum Grenville age²⁸. This evidence for late Precambrian continental basement under the Midland Valley agrees well with the LISPb north–south seismic profile across

Scotland²⁹ and possibly supports both Kennedy³⁰ and George³¹ who found evidence of a southerly source for the Upper Dalradian sediments.

The temporal and spatial setting of the probable post-Grenville quartzite deposition and its metamorphism in the late Precambrian, possibly very early Cambrian time, is critical to the evaluation of the early history of the Midland Valley block. It would, nevertheless, seem likely (also with evidence for Grenville basement under the Dalradian^{32,33}) that the Midland Valley began its separate development after the rifting associated with the extrusion of the Tayvallich volcanics³⁴ which initiated the southward derived sediments of the Upper Dalradian.

The Tayvallich volcanism has been correlated with similar basic volcanism in Newfoundland (dated at 605 $\pm$ 10 Myr³⁵) which has been attributed to the opening of Iapetus³⁶. Our evidence, however, suggests that this rifting event was related to the formation of the first marginal basin along the southern margin of the North American Continent²³.

The serpentinite, spilite, radiolarian chert and black shale assemblages along the Highland Boundary Fault (HBF) are probably of Arenig–Llanvirn age³⁷. The new data make it likely that these rocks were deposited behind the magmatic arc, in a marginal basin. The later closure of this basin, by subduction related under thrusting, then resulted in the preservation of a thin ophiolitic sliver along the HBF (see Fig. 2). This closure is likely to have occurred in Caradocian time, immediately after the deposition of the arenaceous 'molasse' of the Margie series, also preserved along the HBF. The closure resulted in the Highland Border rocks having a structural grain similar to those of the adjacent Dalradian, thus giving a spurious impression of structural unity in them both.

The evidence from the Tayvallich volcanics, the HBF suite and also the Ballantrae Complex³⁸ indicate that the southern margin of the North American continent comprised a series of marginal basins and associated microcontinents. It therefore seems improbable that the Grampian Orogeny resulted from a large-scale continent–continent collision. Consequently we conclude that the landmass which supplied the sediments of the Upper Dalradian was emplaced beneath the latter (see Fig. 2) resulting in the symmetrical folding and mushrooming of the Grampian orogeny and the dramatic uplift along the HBF³⁹.

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- Kuening, P. H. *Konink. Ned. Akad. Wet. Verh., Afd. Natuurk* **20**, 1–47 (1953).
- Hubert, J. F. in *North Atlantic—Geology and Continental Drift* (ed. Kay, M.) 267–282 (American Association of Petroleum Geologists, Tulsa, 1969).
- Williams, A. *Mem. geol. Soc. Lond.* **3**, 265P (1962).
- Dewey, J. F. *Scott. J. Geol.* **7**, 219–240 (1971).
- Phillips, W. E. A., Stillman, C. J. & Murphy, T. J. *geol. Soc. Lond.* **132**, 579–609 (1976).
- Lambert, R. St. J. & McKerrow, W. S. *Scott. J. Geol.* **12**, 271–292 (1976).
- Blaxland, A. B., van Breemen, O., Emeleus, C. H. & Anderson, J. G. *Bull. geol. Soc. Am.* **89**, 231–244 (1978).
- York, D. *Earth planet. Sci. Lett.* **5**, 320–324 (1969).
- Strecheisen, A. *Earth. Sci. Rev.* **12**, 1–33 (1976).
- Ingham, J. K. *Geol. J. Spec. Issue No.* **10**, 115–138 (1978).
- Blatt, H., Middleton, G. & Murray, R. *Origin of Sedimentary Rocks* (Prentice-Hall, Englewood Cliffs, 1972).
- Churkin, M. Jr, Carter, C. & Johnson, B. R. *Geology* **5**, 452–456 (1977).
- Rundle, C. C. *J. geol. Soc. Lond.* **136**, 29–38 (1978).
- Ross, R. J. Jr et al. *U.S. Geol. Surv. Open File Rep.* **78**–701, 363–365 (1978).
- Pankhurst, R. J. *Bull. geol. Soc. Am.* **85**, 345–350 (1974).
- Long, L. E. *J. geophys. Res.* **69**, 1589–1597 (1964).
- Pidgeon, R. T. & Johnson, M. R. W. *Earth planet. Sci. Lett.* **24**, 105–112 (1974).
- Buswiel, M. T., Pankhurst, R. J. & Wadsworth, W. J. *Miner. Mag.* **40**, 363–376 (1975).
- Kistler, R. W. & Peterman, Z. E. *Bull. geol. Soc. Am.* **84**, 3489–3512 (1973).
- Armstrong, R. L., Thorbecke, W. H., & Hales, P. O. *Bull. geol. Soc. Am.* **88**, 397–411 (1977).

21. Moseley, F. *Bull. geol. Soc. Am.* **88**, 764–768 (1977).
22. Kelling, G. J. *geol. Soc. Lond.* **117**, 37–75 (1961).
23. Wright, A. E. *Estudios geol.* **33**, 303–313 (1977).
24. Mitchell, A. H. G. *J. Geol. Chicago* **86**, 643–646 (1977).
25. McGiven, A. thesis, Univ. Glasgow (1967).
26. Upton, B. G. J., Aspen, P., Graham, A. M. & Chapman, N. *Nature* **260**, 517–518 (1976).
27. Graham, A. M. & Upton, B. G. J. *J. geol. Soc. Lond.* **135**, 219–228 (1978).
28. van Breemen, O. & Hawkesworth, C. J. (in the press).
29. Bamford, D., Nunn, K., Prodehl, C. & Jacobs, B. J. *geol. Soc. Lond.* **133**, 481–488 (1977).
30. Kennedy, W. Q. *Trans. geol. Soc. Glasg.* **23**, 107–133 (1958).
31. George, T. N. *Trans. geol. Soc. Glasg.* **24**, 32–107 (1960).
32. Pankhurst, R. J. & Pidgeon, R. T. *Earth planet Sci. Lett.* **31**, 55–68 (1976).
33. van Breemen, O., Halliday, A. N., Johnson, M. R. W. & Bowes, D. R. *geol. J. Spec. Issue No.* **10**, 81–106 (1978).
34. Graham, C. J. *geol. Soc. Lond.* **132**, 61–84 (1976).
35. Stukas, V. & Reynolds, P. H. *Earth planet. Sci. Lett.* **22**, 256–266 (1974).
36. Dewey, J. F. *Nature* **222**, 124–129 (1969).
37. Downie, C., Lister, T. R., Harris, A. L. & Fettes, D. J. *Inst. geol. Sci. Rep.* 71/9 (1971).
38. Bluck, B. J. *Geol. J. Spec. Issue* 151–162 (1978).
39. Harper, C. T. *Scott. J. Geol.* **3**, 46–66 (1967).
40. Jehu, T. J. & Campbell, R. *Trans. R. Soc. Edinb.* **52**, 175–212 (1917).

Fly photoreceptor fluorescence is related to UV sensitivity

THE predominant photoreceptor type in flies, R1-6, contains a rhodopsin which absorbs maximally at around 480 nm. Light converts rhodopsin into a thermostable metarhodopsin peaking at around 580 nm (refs. 1–8). A curious discrepancy is the finding that the electrophysiological sensitivity is highest in the UV^{5,9}—completely at variance with known rhodopsin absorption curves. Several possible explanations of the high UV-peak, including the presence of a UV-rhodopsin and waveguide effects, have recently been excluded^{5–10}. Kirschfeld and co-workers have presented an alternative explanation^{6,11}, that R1-6 also contains a photostable pigment that absorbs UV light and converts the photopigment by energy transfer. This hypothesised sensitising pigment would most probably be a carotenoid, since vitamin A deprivation preferentially suppresses UV sensitivity^{8,12,13}. Of course, vitamin A deprivation also lowers sensitivity by reducing the photopigment level^{14,15}. Here we present further evidence for the existence of a vitamin A-dependent non-photopigment substance in fly photoreceptors. We measured fluorescence while simultaneously monitoring photopigment conversions and argue that the fluorescence is probably related to the UV-sensitivity.

This and previous studies on fly photoreceptors exploited the phenomenon of the deep pseudopupil. The deep pseudopupil is a superposition of the magnified virtual images of the distal photoreceptor endings located in several ommatidia (depending on the numerical aperture of the objective). It is observed by

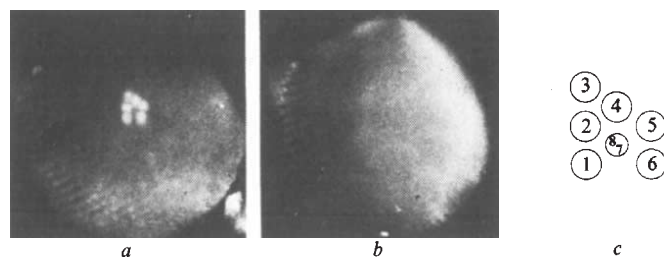


Fig. 1 UV-induced fluorescence photographed in the deep pseudopupil of the compound eye of completely intact and living white-eyed *Drosophila* (mutants *cinnabar* (*cn*) and *brown* (*bw*)), reared on diets high (a) and low (b) in vitamin A. In flies the rhabdomeres, which are the photopigment containing structures of the photoreceptors, are organised in a characteristic pattern (Fig. 1c). Only the rhabdomeres of cells R1-6 of high vitamin A flies (a) give an observable fluorescence to UV excitation in the deep pseudopupil. The vitamin A-dependent rhabdomere emission is pink (>550 nm). However, when superimposed on the blue autofluorescence of the eye, which is not vitamin A dependent, the fluorescent deep pseudopupil in vitamin A enriched flies has a whitish appearance. No distinct fluorescence is observed from the deep pseudopupil of vitamin A deprived flies (b).

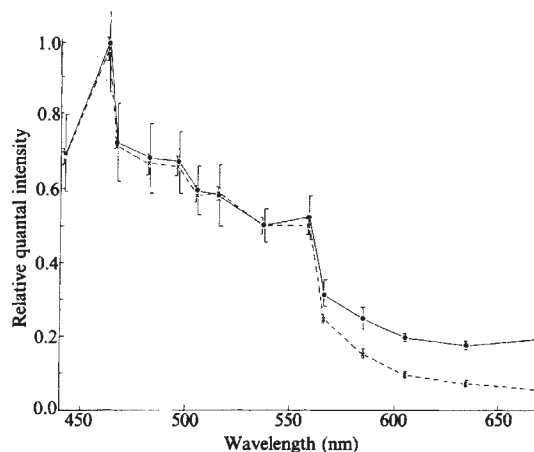


Fig. 2 Emission spectrum for UV excited fluorescence from the deep pseudopupil of *Drosophila*; exciting wavelength 365 nm. The relative quantal emission as a function of wavelength is plotted for high (●) and low (×) vitamin A reared *Drosophila* ($n = 4$ of each type, standard errors are calculated among different preparations).

focusing at the centre of curvature of the eye^{16–18}. Fluorescent deep pseudopupils from white-eyed fruit flies (mutants *cn bw* *Drosophila melanogaster*) under UV excitation are shown in Fig. 1. The six bright spots in Fig. 1a (high vitamin A reared fly) demonstrate that a fluorescing substance is located in the rhabdomeres, that is, the visual pigment containing organelles of the photoreceptors, of cells R1-6. This fluorescing substance is clearly vitamin A dependent, as the fluorescence is absent in flies deprived of vitamin A (Fig. 1b).

The emission spectrum under UV excitation from the deep pseudopupil of flies fed on diets high and low in vitamin A was measured (Fig. 2). The difference, representing the emission spectrum of the vitamin A-dependent substance, is substantial only above 560 nm. Measurements were performed in a Leitz Dialux microscope equipped with incident fluorescence excitation and an EMI 9659 QB photomultiplier. An aperture delimited the area of all six photoreceptor images in the deep pseudopupil ($\approx 80 \mu\text{m}$ diameter on the eye). For technical reasons we were unable to determine the emission spectrum above 670 nm. However, we suppose that the emission spectrum peaks in the red. In the experiments presented below (Figs 2 and 3), we measured fluorescence at >570 nm to accentuate the vitamin A-dependent proportion of the emission.

The magnitude of the emission is virtually identical whether the visual pigment molecules are either predominantly in the rhodopsin or in the metarhodopsin state (Fig. 3). Photopigment conversions were measured by antidromic transmission, that is, illuminating the head capsule, which is rather transparent in *Drosophila*, from the back, and then measuring the light leaving the distal part of the rhabdomeres¹⁹. Visual pigment conversion was monitored at 578 nm, near the peak of the metarhodopsin absorption spectrum ($\lambda_{\text{max}} \approx 580 \text{ nm}$ for *Drosophila*)^{4,5,8,13,20}. At the beginning of the experiment shown in Fig. 3 a high rhodopsin content was produced by a preceding 578 nm light. The transmission at 578 nm then is initially high, but it drops when the eye is irradiated by UV (365 nm) or blue (437 nm) light, due to conversion of rhodopsin into metarhodopsin. On the other hand, however, the fluorescence signal above 570 nm induced by the identical UV and blue irradiation intensities remains constant. This also proves to be the case in a comparable experiment performed on the white-eyed mutant *chalky* of the blowfly *Calliphora erythrocephala* (Fig. 3).

Since fluorescence was independent of whether the photopigment was in the rhodopsin or metarhodopsin state, it is likely that the vitamin A-dependent substance which fluoresces on UV excitation is related to the photoreceptor's UV sensitivity. To characterise this substance its excitation spectrum was found by

determining the light intensity needed to evoke a criterion emission >570 nm as a function of excitation wavelength. The spectral characteristics of the vitamin A-dependent substance were factored from background measuring from the deep pseudopupil of both high and low vitamin A reared *Drosophila*. A spectrum peaking in the UV with a tail in the blue was obtained (Fig. 4). (This tail could result from the long wavelength fluorescence which has been characterised for R7, another photoreceptor type, under blue excitation^{21,22}.) The nature of the fluorescing substance, of course, is still enigmatic, except for its vitamin A dependence. Nevertheless, in principle it qualifies well for the hypothesised sensitising pigment which functions in the visual process by transferring energy to both rhodopsin and metarhodopsin^{5,6,8,11,21}.

Note that a distinct red fluorescence under blue excitation was observed in R1-6 receptors of the housefly *Musca* by Franceschini^{21,22}, which was interpreted to be related to the amount of metarhodopsin. The data shown in Fig. 2 argue against this interpretation since fluorescence does not change during a monitored rhodopsin to metarhodopsin conversion. We have further shown that in *Drosophila* red fluorescence is only induced with blue excitation intensities 2–3 log units above those applied in the experiment of Fig. 3 (as well as for Fig. 4). Such extremely intense blue irradiation ($\approx 10^{18}$ quanta $\text{cm}^{-2} \text{s}^{-1}$) also causes photolysis of the visual pigment. However, Franceschini has categorised different photoreceptor types by observations of this bright light induced fluorescence and we do not challenge his classification scheme^{21,22}.

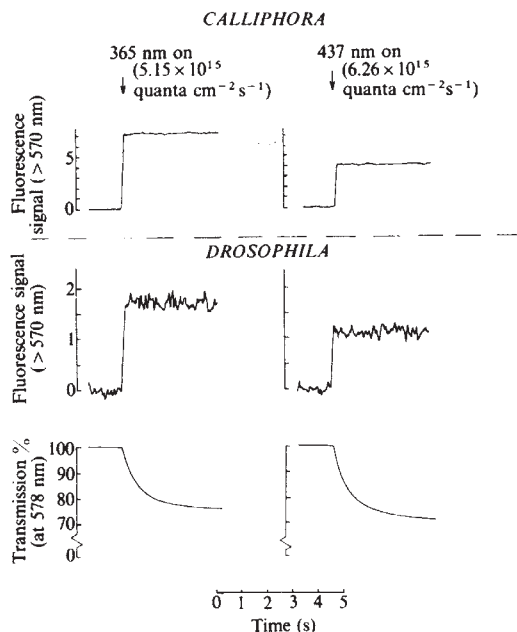


Fig. 3 Fluorescence and antidromic transmission measurements from the fly deep pseudopupil. Rhabdomere transmission was measured by illuminating the back of the rather transparent head of a *Drosophila* (that is antidromically) and determining the relative light intensity leaving the distal rhabdomere tips as visualised in the deep pseudopupil¹⁹. Yellow light of 578 nm wavelength was used to detect conversion of blue absorbing rhodopsin into yellow absorbing metarhodopsin induced by irradiating the eye with UV (365 nm) and blue (437 nm) light. (Initially a photoequilibrium with a high rhodopsin fraction was established by prolonged 578 nm light.) The indicated intensities were selected to induce conversion taking of the order of seconds. The fluorescence induced by these same intensities stays constant during this time period, and thus is independent from the state of the visual pigment molecules. The identical conclusion is obtained in the case of a white-eyed mutant (*chalky*) of the blowfly *Calliphora*. Vitamin A-enriched flies were used. From measurements on vitamin A deprived *Drosophila* it follows that background fluorescence due to eye tissue amounts to about 0.9 divisions in both cases of irradiation.

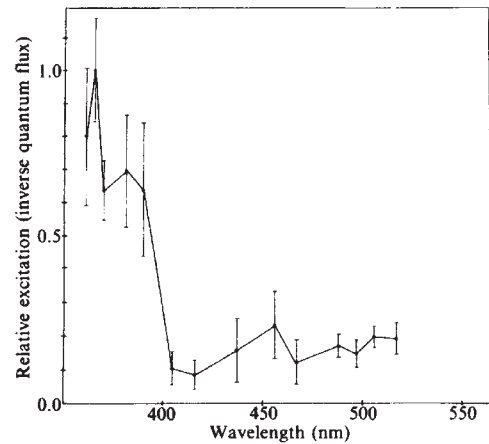


Fig. 4 Excitation spectrum of the vitamin A-dependent photo-stable substance of *Drosophila* photoreceptors. The intensities $I_h(\lambda)$ and $I_l(\lambda)$ needed to evoke a criterion signal c of emitted light >570 nm were measured in the case of high and low vitamin A reared flies respectively. (The identical aperture around the centre of the deep pseudopupil, enclosing all photoreceptor types, was applied throughout the experiments.) Let the spectral excitation of the vitamin A-dependent substance and that of the background be given by ϵ_s and ϵ_b respectively. Then it follows from $c = I_h(\epsilon_s + \epsilon_b)$ and $c = I_l\epsilon_b$ that $\epsilon_s \propto (I_h^{-1} - I_l^{-1})$. The assumed linearity of fluorescence signal with intensity was tested and verified. The data are from four high and four low vitamin A preparations with standard errors of difference shown.

It was claimed recently²³ that changes in the antidromic transmission caused by high intensities ($\approx 5 \times 10^{15}$ quanta $\text{cm}^{-2} \text{s}^{-1}$) of blue light are too slow (minimum time constant >40 ms) to reflect visual pigment conversions. This is contrary to our interpretation of the Fig. 2 data. However, an extensive body of literature^{2,3,5,18,24,25} is entirely consistent with the interpretation that these transmission changes are mediated exclusively by photopigment conversions. Moreover, we did not confirm the slow change in transmission²³ in our measurements of antidromic transmission from the deep pseudopupil of completely intact and living *Drosophila*. The time constant for the transmission change reflecting the speed of rhodopsin to metarhodopsin conversion was directly measured by applying light from a pulsed blue dye laser. The time constant is ≈ 90 μs , confirming predictions made on the basis of low temperature camera-flash experiments²⁶.

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- Hamdorf, K., Paulsen, R. & Schwemer, J. in *Biochemistry and Physiology of Visual Pigments* (ed. Langer, H.) 155–166 (Springer, Berlin, 1973).
- Stavenga, D. G., Zantema, A. & Kuiper, J. W. in *Biochemistry and Physiology of Visual Pigments* (ed. Langer, H.) 175–180 (Springer, Berlin, 1973).
- Stavenga, D. G., *J. comp. Physiol.* **111**, 137–152 (1976).
- Ostroy, S. E., Wilson, M. & Pak, W. L. *Biochem. biophys. Res. Commun.* **59**, 960–966 (1974).
- Stark, W. S., Frayer, K. L. & Johnson, M. A. *Biophys. Struct. Mech.* **5**, 197–209 (1979).
- Kirschfeld, K. *Biophys. Struct. Mech.* **5**, 117–128 (1979).
- Tsukahara, Y. & Horridge, G. A. *J. comp. Physiol.* **114**, 233–251 (1977).

8. Stark, W. S., Ivanyshyn, A. M. & Greenberg, R. M. *J. comp. Physiol.* **121**, 289–305 (1977).
9. Burkhardt, D. *Symp. Soc. exp. Biol.* **16**, 86–109 (1962).
10. Kirschfeld, K. & Snyder, A. W. *Vision Res.* **16**, 775–778 (1976).
11. Kirschfeld, K., Franceschini, N. & Minke, B. *Nature* **269**, 386–390 (1977).
12. Goldsmith, T. H. & Fernandez, H. R. in *The Functional Organization of the Compound Eye* (ed. Bernhard, C. G.) 125–143 (Pergamon, Oxford, 1966).
13. Stark, W. S., Ivanyshyn, A. M. & Hu, K. G. *Naturwissenschaften* **63**, 513–518 (1976).
14. Razmjoo, S. & Hamdorf, K. *J. comp. Physiol.* **105**, 279–286 (1976).
15. Harris, W. A., Ready, D. F., Lipson, E. D., Hudspeth, A. J. & Stark, W. S. *Nature* **266**, 648–650 (1977).
16. Franceschini, N. & Kirschfeld, K. *Kybernetik* **9**, 159–182 (1971).
17. Franceschini, N. in *Photoreceptor Optics* (eds Snyder, A. W. & Menzel, R.) 98–125 (Springer, Berlin, 1975).
18. Stavenga, D. G. in *Handbook of Sensory Physiology* Vol. VII/6A (ed. Autrum, H.) 357–439 (Springer, Berlin, 1979).
19. Kirschfeld, K. & Franceschini, N. *Kybernetik* **5**, 47–52 (1968).
20. Harris, W. A., Stark, W. S. & Walker, J. A. *J. Physiol., Lond.* **256**, 415–439 (1976).
21. Franceschini, N. *Neurosci. Lett. Suppl.* **1**, S, 405 (1978).
22. Franceschini, N. *Proc. int. Un. Physiol. Sci.* **13**, 237 (1977).
23. Lo, M.-V. C. & Pak, W. L. *Nature* **273**, 772–774 (1978).
24. Muijser, H. & Stavenga, D. G. *Biophys. Struct. Mech.* **5**, 187–196 (1979).
25. Stavenga, D. G. *Biophys. Struct. Mech.* **5**, 175–185 (1979).
26. Kirschfeld, K., Feiler, R. & Minke, B. *Z. Naturforsch.* **33c**, 1009–1010 (1978).

Saccadic suppression by corollary discharge in the locust

DURING rapid eye movements, saccades, human subjects do not perceive movement of their visual surroundings, and their sensitivity to light flashes and moving objects is reduced. This phenomenon is known as saccadic suppression. The physiological basis of this suppression is not well understood. The necessity for a mechanism which allows discrimination between retinal image movement generated by the object and that generated by the subject was recognised by Helmholtz¹. Sperry² proposed that along with the eye movement, the central nervous system generates a 'corollary discharge' into the visual centres that compensates for displacement of the retinal image, and von Holst and Mittelstaedt³ independently postulated a similar 'efference copy' mechanism. Functionally significant efferent control has been demonstrated in several non-visual sensory pathways^{4,5}. Such a mechanism could account for experimentally produced effects in human subjects^{6,7}. The prevailing view^{8–12}, however, is that saccadic suppression in higher mammals is caused not by corollary discharge but instead by inhibition derived from the retinal image motion generated by eye movement. This view has been extended to include saccadic suppression of the visual movement detector system in crickets and locusts^{13–15}, in which the neural circuit performing the inhibitory function has been identified^{15,16}. The experiments leading to this conclusion have all used passive movement of the animal with respect to the visual environment, or the reverse, and not true saccades. We have now investigated the effects of head movements by the locust on its visual responses, and report that movement detector neurones are strongly inhibited during the fast phase of optokinetic nystagmus and during voluntary saccades by a central nervous mechanism of the corollary discharge type. The effect of the inhibitory mechanisms of retinal origin are negligible by comparison.

In the locust movement detector system, one can study the neurophysiology of saccadic suppression in identified neurones that have well understood feature-detection properties. The lobula giant movement detector neurone and its tightly coupled postsynaptic cell, the descending contralateral movement detector (DCMD) neurone (the identified neurones of the locust movement detector system) are optimally responsive to novel, small-field stimuli in their hemispherical visual fields^{14–18}. The DCMD neurones provide synaptic input to identified metathoracic motorneurones used for escape jumping^{19,20} that can be provoked by novel changes in contrast. Locusts do not have moveable eyes and perform saccadic movements by turning their heads rapidly²¹. The changes in visual input thereby produced could inappropriately excite the DCMD neurones. This possibility is greatly reduced by the inhibition reported here that accompanies the fast phase of optokinetic nystagmus and voluntary saccades.

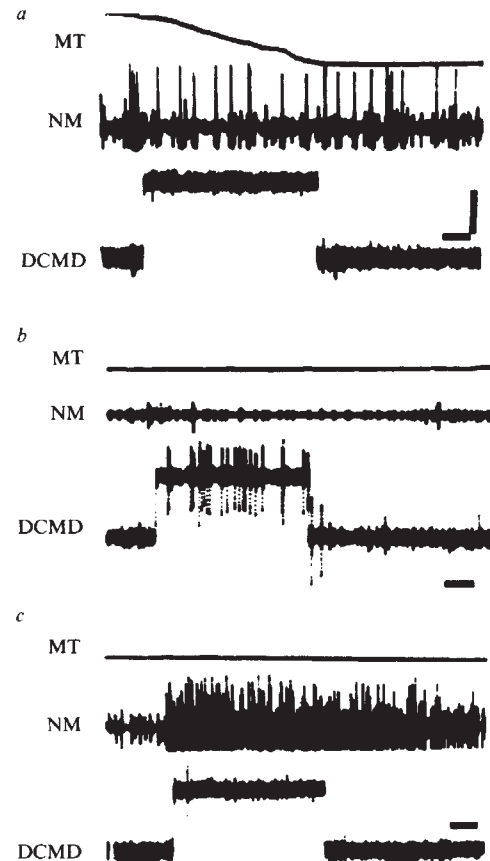


Fig. 1 Inhibition of the response of the DCMD to a moving spot stimulus during the fast phase of optokinetic nystagmus. *a*, A fast flick-back saccade of the locust's head is indicated by the abrupt decrease in the level of the signal produced by a capacitive movement transducer (MT)³⁴. The onset of a burst of spikes of an M51 fast unit (NM) precedes the onset of the saccade by a brief interval. The high rate of firing of the M51 fast unit, triggers the stimulus, a moving spot of light produced by an oscilloscope beam, visible to the locust through a 15° window in the striped drum. The duration of the moving spot stimulus is indicated by the voltage step superimposed on the DCMD record (bottom trace). The DCMD neurone recording shows that there is no response to the moving spot stimulus during a fast flick-back. *b*, Thirty seconds after each saccade-triggered moving spot, a control stimulus, a second moving spot of light, similar to the experimental stimulus, excites the movement detector system in the absence of fast head turning, during the slow (following) phase of optokinetic nystagmus. The fast M51 unit (MN) is silent, and the DCMD responds vigorously to a moving spot stimulus. *c*, The locust's head is rigidly clamped to its prothorax, giving a constant value of the MT signal. The high frequency firing of M51 (NM) triggers a moving spot stimulus. The DCMD response is strongly suppressed during the M51 burst. Movement transducer calibration (vertical bar): 20°. Time scale (horizontal bar), 100 ms.

In the absence of movement of the visual field, locusts produce voluntary saccades, but they do so rarely. The fast phase of optokinetic nystagmus and voluntary saccades follow the same amplitude-duration relationship in locusts²¹, and they are regarded as identical in higher mammals²². In order to make a large number of observations, therefore, saccadic movements were evoked in our experiments by tethering locusts inside a rotating striped drum, whereupon they readily perform optokinetic nystagmus, consisting of a slow (following) phase and a succeeding fast (flick-back) phase. Locusts were suspended by their pronotum, given a polystyrene ball to hold with their legs and left otherwise free to move. Single unit action potentials were recorded from muscle 51 (M51) of the neck, one of the muscles which turns the head²³, and from the ventral nerve cord axon of the DCMD by means of chronically implanted electrodes made from 100-μm insulated silver wire.

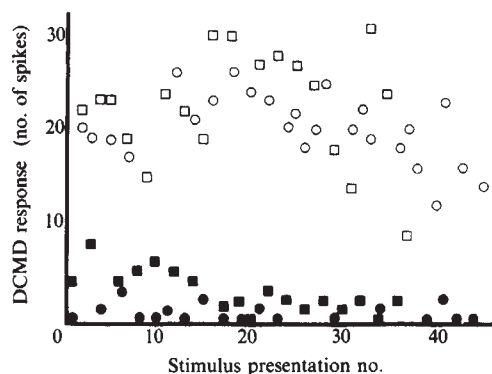


Fig. 2 The responses of the DCMD to two sequences of moving spot stimuli, recorded from (1) an open-loop condition locust (squares) and (2) from a closed-loop condition locust (circles). In both sequences experimental stimuli (solid symbols), triggered by fast-flick back motor commands, are followed 30 s later by control stimuli (open symbols), during which there is no flick-back command. Precautions were taken throughout all stimulus presentations to insure that a reduction of the DCMD response during saccades is a consequence of inhibition and not one of habituation of the response of the DCMD to repetitive stimuli²⁴. A gating circuit effected a long minimal interval, 1 min, between successively triggered moving spot stimuli, and the control stimulus followed the experimental stimulus, rather than preceded it. The target stimulus was moved on the face of the oscilloscope by two independent, free-running waveform generators of slightly different frequency applied to the *x* and *y* deflection plates, so that it rarely traversed the same path twice.

The response of the DCMD to small moving stimuli is easily recognised as a burst of large single-unit action potentials delayed with respect to the stimulus by 50–75 ms (refs. 14, 17). During the fast phase of optokinetic nystagmus or during spontaneous saccades when the drum is stationary, the response of the DCMD is strongly inhibited; it responds not at all or with just a few action potentials (Figs 1a, 2). During the slow phase of optokinetic nystagmus or if the locust's head is stationary, the DCMD responds vigorously to optimal stimuli (Figs 1b, 2).

Activity of the fast and slow motor axons which excite M51 can be identified in extracellular recordings from the fibres of M51 as large (fast units) and small (slow units) spikes, respectively²³. We find that inhibition of the DCMD is strongly correlated with high frequency burst activity of the fast motor units of M51 (Fig. 1a, c), and that the DCMD is not inhibited during low frequency tonic activity of either fast or slow M51 motor units (Fig. 1b). DCMD inhibition does not terminate sharply with the end of M51 fast motor unit bursts, but may continue afterwards for a duration of 200–300 ms. High frequency antidromic stimulation of the motoneurons to M51 does not produce inhibition of the DCMD. Inhibition of the DCMD is thus correlated with commands for fast turning of the head and not simply with activity of fast M51 motor units.

Relative movement of the eye and a complex visual background can suppress the response of the locust movement detector system^{24,25} by mechanisms that are visual and not efferent in origin^{15,16,18}. The inhibitory influence of the rotating striped drum in these experiments can be discounted. The contrast frequency, 0.02 Hz, produced by rotation of the striped drum alone is far too low to produce inhibitory effects of visual origin¹⁵. However, visual mechanisms for the inhibition of the locust movement detector system might well be activated and cause the inhibition we have described during the fast phase of optokinetic nystagmus when the locust's head turns with angular velocity as high as 300 deg s⁻¹. This possibility is excluded by two lines of evidence. First, the DCMD is strongly inhibited during spontaneous commands for voluntary saccades in a homogeneous white background. And second, after the head has been rigidly secured to the body (open-loop condition), the unilateral high frequency burst activity of the fast motor units of M51 which would normally cause rapid head movement still

coincides with a period of strong inhibition of the DCMD response (Figs 1c, 2). This experiment also precludes proprioceptive feedback from head movement, via cervical and proprioceptive hairs^{26,27} as a source of inhibition. (Elimination of proprioceptive feedback by securing the locusts head to its body or by elimination of neck hairs²¹ reduces the frequency of occurrence of fast flick-backs but does not abolish them, contrary to Shepherd's²³ interpretation.

Further experiments, in which the nerves supplying the neck muscles are cut and motor unit activity recorded from the cut ends of the motor axons to M51, exclude proprioceptive feedback from neck muscle stretch receptors (should these exist) as a source of inhibition of the locust movement detector system during the fast phase of optokinetic nystagmus and voluntary saccades. The DCMD is as strongly inhibited in these conditions as it is when the neck muscles can contract. A command for rapid head turning is therefore a sufficient condition for inhibition of the locust movement detector neurones. Neither change in visual contrast, nor proprioceptive information produced by change in position of the head or muscle contraction, nor the visual signal produced by the rotating striped drum used to induce optokinetic nystagmus contribute significantly to this inhibition, which must therefore be efferent in origin and distinct from the previously described^{15,16,18} visual inhibitory mechanisms of the locust movement detector system.

Relative movement of the eye and environment thus inhibits the locust movement detector neurones by either central or peripheral visual mechanisms. Presumably the same is true for the cricket movement detector system which is very similar to that of the locust^{13,14}. These mechanisms do not effect inhibition of the entire locust visual system. For example, visual interneurons that are excited by relative movement of eye and background are not suppressed during optokinetic nystagmus²⁸. The movement detector neurones, however, are inhibited predominantly by corollary discharge during saccadic movements, but visual reafference could function alone when movement is otherwise produced as in flight or locomotion. Central inhibitory mechanisms may be active here, as well.

Saccadic suppression in mammalian visual systems may involve mechanisms of both central and peripheral origin. However, there has been no neurophysiological demonstration of a functionally significant corollary discharge mechanism for saccadic suppression in any vertebrate, such as we have shown occurs in the locust. The similarities between the fast phase of optokinetic nystagmus in the locust and mammalian saccades^{29,30}, the former a continuous movement with a small amount of jitter, duration typically 200–300 ms and angular velocity maximally 300 deg s⁻¹ (ref. 21), suggest that corollary discharges may at least participate in saccadic suppression of mammalian visual systems. A striking example of inhibition produced by corollary discharges associated with saccadic movements is found in the visual neurones of the superficial layer of the superior colliculus of the rhesus monkey^{31,32,35}. Although they are in themselves poor sensory analysers, anatomical pathways exist³³ by which the suppression of the activity of the neurones of the superior colliculus could affect perceptual function through modification of the responses of the feature detector units of striate and extra-striate visual cortex. As in the locust, visual perception in higher mammals could thus be inhibited by corollary discharges that accompany saccadic movements as well as by retinal image motion^{8–10,12}.

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1. Helmholtz, H. von *Physiological Optics* Vol. III (ed. Southall, J. P. C.) (Optical Society of America, Menasha, Wisconsin, 1925).
2. Sperry, R. W. *J. comp. Physiol. Psych.* **43**, 482–489 (1950).
3. von Holst, E. & Mittelstaedt, H. *Naturwissenschaften* **37**, 464–476 (1950).
4. Kuffler, S. W., Hunt, C. C. & Quilliam, J. P. *J. Neurophysiol.* **14**, 29–54 (1951).
5. Flock, A. & Russell, I. J. *J. Physiol., Lond.* **235**, 591–605 (1973).
6. Teuber, H.-L. in *Handbook of Physiology*, section I. Vol. 3 (ed. Field, J.) 1595–1668 (American Physiological Society, Washington, 1960).
7. Riggs, L. A., Merton, P. A. & Morton, H. B. *Vision Res.* **14**, 997–1011 (1974).
8. Wurtz, R. H. *J. Neurophysiol.* **32**, 975–986 (1969); **32**, 987–994 (1969).
9. Noda, H. *J. Physiol., Lond.* **249**, 87–102 (1975); **250**, 579–595 (1975).
10. Judge, S. J. & Wurtz, R. H. *Soc. Neurosci. 7th A. Meet. Abs.* **3**, 564 (1977).
11. Matin, E. *Psychol. Bull.* **81**, 899–917 (1974).
12. Wurtz, R. H. & Campbell, F. W. *Ass. Res. Vision Ophthalmol., Spring Meeting*, 1977, p. 106.
13. Palka, J. *J. exp. Biol.* **50**, 723–732 (1969); *Am. Zool.* **12**, 497–505 (1972).
14. Palka, J. *J. Insect Physiol.* **13**, 235–238 (1967).
15. Rowell, C. H. F., O'Shea, M. & Williams, J. L. D. *J. exp. Biol.* **68**, 157–185 (1977).
16. O'Shea, M. & Rowell, C. H. F. *Nature* **254**, 53–55 (1975); *J. exp. Biol.* **65**, 389–408 (1976).
17. Rowell, C. H. F. *Z. vergl. Physiologie* **73**, 167–194 (1971).
18. Rowell, C. H. F. & O'Shea, M. *J. exp. Biol.* **65**, 273–288 (1976).
19. Burrows, M. & Rowell, C. H. F. *J. comp. Physiol.* **85**, 222–234 (1973).
20. Pearson, K. & Goodman, C. *J. comp. Neurol.* **184**, 141–166 (1979).
21. Kien, J. & Land, M. F. *Physiological Entomol.* **3**, 53–57 (1978).
22. Wallace, M., Blair, S. M. & Westheimer, G. *Expl Brain Res.* **33**, 19–25 (1978).
23. Shephard, P. *J. exp. Biol.* **60**, 735–768 (1974).
24. Horn, G. & Rowell, C. H. F. *J. exp. Biol.* **49**, 143–169 (1968).
25. Palka, J. *Am. Zool.* **7**, 7–28 (1967).
26. Haskell, P. T. *Nature* **183**, 1106–1107 (1959).
27. Goodman, L. J. *J. exp. Biol.* **42**, 385–407 (1965).
28. Kien, J. *J. comp. Physiol.* **113**, 161–180 (1977).
29. Collewijn, H. *Vision Res.* **9**, 803–814 (1969).
30. Robinson, D. A. *J. Physiol., Lond.* **174**, 245–264 (1964).
31. Robinson, D. L. & Wurtz, R. H. *J. Neurophysiol.* **39**, 852–870 (1976).
32. Richmond, B. J. & Wurtz, R. H. *Soc. Neurosci. 7th A. Meet. Abstr.* **3**, 574 (1977).
33. Benvenuto, L. A. & Rezak, M. *Brain Res.* **108**, 1–24 (1976).
34. Sandeman, D. C. *Comp. Biochem. Physiol.* **24**, 635–638 (1968).
35. Westheimer, G. *Archs Ophthalmol.* **52**, 710–724 (1954).

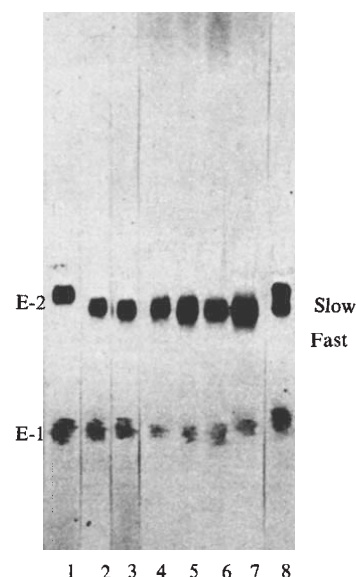


Fig. 1 Esterases of larvae obtained from eggs supplemented with blastulae nuclei. Individual larvae 120 h old were homogenised in a 1% solution of Triton X-100 in distilled water. Each homogenate was centrifuged (2,000g per 10 min) and aliquots of supernatant were subjected to polyacrylamide gel disc electrophoresis in 1-mm diameter tube. Esterase bands were identified using a slightly modified method of Sims¹⁰. The substrate was α -naphthylacetate. The start is at the top and the anode is at the bottom. Two zones of esterase activity are revealed, the more cathodal being E-2 carboxylesterase. Gels 1 and 2 show the mobility of two allelic variants of E-2 slow and fast isoenzymes in homozygous larvae, respectively. Gel 8 results from a control mixture made from the homogenate analysed in gel 3 and from a standard slow E-2 preparation.

Nuclear transplantation in teleost *Misgurnus fossilis* L.

ALTHOUGH the transplantation of somatic cell nuclei into eggs has been achieved in amphibian species^{1,2}, *Drosophila*³ and some mammals^{4,5}, it is important to use further groups of animals because experience with amphibia has shown that the subsequent development of normal animals depends largely on the species used. We report here successful transplantation of nuclei from middle or late blastula into the eggs of teleost fishes. The eggs of teleosts have several distinctive features of structure, cleavage and early development which make nuclear transplantation more difficult than with amphibia. Teleost eggs are telolecithal, with the yolk segregated from the active cytoplasm. We used eggs and embryos of *Misgurnus fossilis* L. (loach). In this fish, 30–60 min after natural or artificial activation of an egg, part of the cytoplasm migrates to the animal pole, forming a small polar cap where the nucleus is situated, completing meiotic division⁶. Thus, nuclei must be introduced into the animal pole region which occupies not more than one-tenth of the total egg.

The procedure was basically similar to that of Briggs and King¹. Eggs of *M. fossilis* L. were obtained by injection of females with chorionic gonadotropic hormone (100 mequiv. per 100 g body weight). They were activated by placing them in Petri dishes with tap water; activated eggs became attached by their vegetal poles to the bottom of the dish. After 30 min, when the polar cap had formed, the water was replaced by Niu-Twitty solution, which served as a medium for further experiments. A blastodisk of mid-blastula stage was removed from the yolk and placed in Niu-Twitty solution without Ca^{2+} and Mg^{2+} . Dissociation was complete after 5–10 min. A single cell ($\sim 50 \mu\text{m}$ in diameter) was sucked into a micropipette of inner diameter about $35 \mu\text{m}$. The end of the pipette was inserted into the egg at

Table 1 Nuclear transplantations in *Misgurnus fossilis* L.

Type of transfer	Total no. of transfers	Cleavages yielding blastulae	No. of blastulae under observation	No. of embryos that reached				
				Gastrula	Blastopore closing	Heartbeat	Prelarvae	Feeding larvae
From mid-blastula to enucleated (by X-irradiation, 15.20 rads) eggs	791	349 ⁺	89(a)	52	29	16	13	10
			146(b)	88			13	
From mid-blastula to enucleated (by X-irradiation, 38–49.40 rads) eggs	72	30	16	9	4	2	2	2
From mid-blastula to non-enucleated eggs	320	156 ⁺	57(a)	30	11	9	7	3
			51(b)	21			6	
Control*	1,000	0	0	0	0	0	0	0

* Irradiated (by X rays) or non-irradiated eggs; both have been activated with tap water.

[†] Some blastulae were used for chromosome analysis and the rest were used for further observation (a) or to provide prelarvae for esterase analysis (b) (Fig. 1).

Table 2 Ploidy estimation in blastulae and gastrulae obtained

Nuclear transfers from mid-blastulae	Stage	Embryos analysed Number	No. of embryos				
			Anucleate subhaploid	Haploid	Diploid	Triploid	Tetraploid
To enucleated eggs	Blastula	30	11	0	16	0	2
	Gastrula	9	0	0	9	0	0
To non-enucleated eggs	Blastula	19	3	2	11	1	2
	Gastrula	6	0	0	5*	1	0
Control	Blastula	7	0	0	7	0	0

Blastodisks isolated from the yolk were fixed in a mixture of alcohol and acetic acid in the ratio 1:3 for 24 h, washed with 70% at 5 °C and stained with acetorcin. Stained blastodisks were squashed in a drop of 45% acetic acid. In each blastodisk preparation 8 to 10 metaphase plates were chosen to calculate chromosome number. The diploid set in *M. fossilis* L. consists of 100 chromosomes⁸. The number of chromosomes in preparations of metaphase plates was counted to an accuracy of $\pm 10\%$, as shown by the analysis of untreated diploid embryos. We therefore took as diploids all treated embryos in which we counted 100 ± 10 chromosomes. Triploid and tetraploid embryos were those with 150 ± 15 or 200 ± 20 chromosomes, respectively. Embryos were termed anucleate or subhaploid if most of the cells had no nuclei and if the few cells which contained nuclei were subhaploids. In one of two blastulae presented above as haploids we counted 50 chromosomes in each metaphase plate analysed and in another 50 ± 5 chromosomes. * Some metaphase plates in these embryos contained a subhaploid (< 20) or polyploid (> 200) number of chromosomes.

equatorial level and the contents were injected into the polar cap region. The operation was carried out within 60 min of activation. Operated eggs were incubated in the same medium at 16–18 °C for 10 days, sufficient for the formation of actively feeding larva.

Two series of experiments were carried out, with and without enucleation of eggs. Enucleation was achieved by X-ray irradiation in an appropriate dose to inactivate the nuclei of loach eggs⁷. As our results show (Table 1) irradiation produced almost complete inactivation of the female pronucleus, while the cytoplasm remained able to support normal development at least to the larval stage. We used 791 enucleated and 320 non-enucleated eggs, and into each of them we transplanted a single somatic cell nucleus (together with cytoplasm) from the mid-blastula. Of the 200 gastrulae thus obtained, 41 developed to the prelarval stage or into actively feeding larvae. The development of most embryos stopped at some point before these stages and in many cases there were evident abnormalities. Some embryos were lost after the feeding larva stage, but others were quite normal and were either used for analysis of enzymes or fixed as total preparations. Unfortunately, we have not been able to maintain the development of loach larvae beyond the stage of actively feeding larva in laboratory conditions.

To investigate whether this embryonic development was determined by the transplanted nuclei, we looked at the karyotypes and carboxylesterase (EC 3.1.1.1.) isoenzymes of the embryos. Table 2 shows that most of the blastulae were diploid, although some were tetraploid. This was possible only if the transplanted nuclei had participated in the development of the embryos. During transplantation some cytoplasm, including the centriole, is transferred from the somatic cell into the egg together with the nucleus. Thus, it might be said that the centriole provokes 'parthenogenetic' cleavage; however, if this were so, only blastulae with an abnormal chromosome set should be formed. Indeed, when enucleated eggs are fertilised with irradiated sperm and are supplemented with sperm centrioles, they begin to cleave but yield only embryos with abnormal chromosome sets, developing no further than the blastula stage.

Transplantation into non-enucleated eggs yielded about the same proportion ($\sim 50\%$) of cleaving embryos which formed blastulae, and about 10% developed into normal larvae. This could not have happened without the transplanted nuclei because unfertilised and artificially activated eggs of *M. fossilis* L. never cleave. Again, it might be said that cleavage was provoked by the introduction of the somatic cell's centriole, but if so, the embryos would have been haploid, for when fertilised with irradiated sperm, loach eggs cleave but spontaneous diploidisation of a female pronucleus does not usually occur and only haploids are formed⁷. Table 2 shows the tentative estima-

tion of the ploidy of blastula cells. We expected that they would be either mainly haploid (if parthenogenetic development was induced by the introduced centriole) or triploid due to fusion of the transplanted nucleus with the female pronucleus. Most embryos seemed to be diploids, with only a small part of the blastulae having cells with $1n$ chromosomes (or even fewer).

Two alleles for carboxylesterase are known in *M. fossilis* L.⁹, coding for two distinct forms of the enzyme (E-2), electrophoretically fast and slow (Fig. 1, gels 1 and 2). Maternal E-2 synthesised at oogenesis has been shown to disappear within 120 h, after which only the esterase controlled by the larval genome is found⁹. When nuclei of blastulae homozygous for fast E-2 were transplanted into enucleated eggs from females homozygous for slow E-2, only fast E-2 was found in the larvae that developed (Fig. 1, gel 3). When nuclei from blastulae homozygous for fast E-2 were transplanted into non-enucleated eggs from blastulae homozygous for slow E-2, five larvae out of six obtained had only fast E-2 (Fig. 1, gels 4–6); in one larva there was a trace of slow E-2 in addition to fast E-2 (Fig. 1, gel 7), indicating partial function of E-2 genes characteristic of the female pronucleus. Probably, either the whole pronucleus or the chromosome carrying the relevant gene was preserved in this larva. Only the blastulae and gastrulae were karyotyped and most of them seemed to be diploid, although there were polyploid individuals among them. Based on these data we consider that our larvae were mainly diploids, and that in the experiments with non-enucleated eggs the female pronucleus was eliminated in the embryos, although it may have been preserved in some cases, yielding triploids, as reported for amphibia¹¹. The possibility that we measured some maternal enzyme activity can be discounted because maternal E-2 esterase synthesised at oogenesis disappears within 120 h (ref. 9) and our analyses were made on larvae older than 120 h. Therefore, we applied an enzyme analysis to indicate that there was little or no activity of the genes of recipient eggs in the resultant larvae.

This cannot be attributed to the fact that cytoplasm of the blastula cell which contained some amounts of donor-like enzyme was introduced to the recipient egg because this cytoplasm constituted less than 0.1% of the egg cap cytoplasm¹² and the exogenous enzyme was further diluted during cell multiplication.

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- Briggs, R. & King, T. *Proc. natn. Acad. Sci. U.S.A.* **38**, 455–463 (1952).
- Gurdon, J. B. *The Control of Gene Expression in Animal Development* (Clarendon, Oxford, 1974).
- Illmensee, K. *Wilhelm Roux Arch. Entw.Mech. Org.* **170**, 267–298 (1972).
- Bromhall, J. D. *Nature* **258**, 719–722 (1975).
- Modlinski, J. *Nature* **273**, 466 (1978).
- Kostomarov, A. A. *The objects of Developmental Biology* (ed. Detlaff, T. A.) (Nauka, Moscow, 1975).
- Neyfakh, A. A. *Trudy IMJ Acad. Sci. USSR* **24**, 135–159 (1959).
- Raicu, P. & Taisescu, E. *J. Hered.* **63**, 92–94 (1972).
- Ivanenkov, V. V. *Carboxylesterase-2 in the Development of the Loach (Misgurnus fossilis L.)* (in the press).
- Sims, M. *Nature* **207**, 757–758 (1965).
- Subtelny, S. & Bradt, C. J. *Morph.* **112**, 45–59 (1963).
- Rott, N. N. & Sheveleva, G. A. *J. Embryol. exp. Morph.* **20**, 141–150 (1968).

Expression of genetic resistance to an oncogenic herpesvirus at the target cell level

THE oncogenic potential of herpesviruses has been demonstrated in several species and herpesviruses have been strongly implicated as aetiological agents of various human neoplastic diseases^{1,2}. The extreme heterogeneity of human populations makes genetic analysis of mechanisms of host resistance to neoplastic disease in man a difficult task. Another herpesvirus, Marek's disease virus (MDV), is the aetiological agent of a naturally occurring lymphoma of chickens, Marek's disease (MD) (for review see ref. 3). MD is the animal model for Burkitt's lymphoma of man in which the Epstein-Barr virus (EBV) has been strongly implicated⁴. At least two loci are associated with resistance to MD: the major histocompatibility complex (MHC)^{5,6} and a non-MHC locus⁷. The mechanism of MHC-associated resistance may involve an active rejection of MD tumour cells⁵. We now report data which strongly suggest that non-MHC-associated genetic resistance is expressed at the level of the target cell for malignant transformation.

The target cell for neoplastic transformation by MDV is generally thought to be a T lymphocyte⁸. The tentative association of the *Ly-4* locus, which codes for alloantigenic markers present on T cells, with the degree of susceptibility to MD⁷ led us

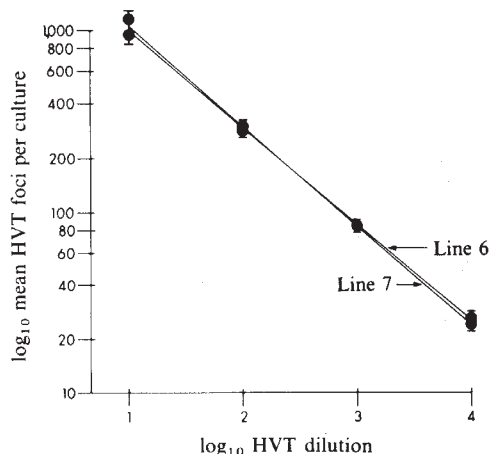


Fig. 1 Secondary chick embryo fibroblasts (CEF) were prepared from 10-day line-6 and line-7 embryos and propagated in Linbro multi-well trays. Lyophilised FC126 strain HVT vaccine was reconstituted with medium and centrifuged at 500g for 15 min to remove large cell debris. Four replicate wells were infected with each serial 10-fold dilution of this preparation, the highest concentration of which had yielded 1.25×10^4 focus-forming units per well when assayed previously on Hy-line SC CEF. HVT foci were stained with Giemsa stain and counted at 64 h post-infection. Error bars indicate 1 s.d. from the mean.

Table 1 Infectious centres induced by spleen cells exposed to HVT *in vitro* or MDV *in vivo*

Spleen cell source	HVT lesions per 1×10^7 cultured spleen cells	MDV lesions per 5×10^5 injected spleen cells
Line 6	33.5 ± 25.5	9.4 ± 6.5
Line 7	223.9 ± 38.0	38.6 ± 3.6

For determination of infectious centres after *in vitro* exposure to HVT, aliquots of the spleen cells used for the HVT adsorption studies of Fig. 2 were washed three times in medium and cultured in RPMI-1640 with 10% fetal calf serum for 24 h at 37°C at a density of 5×10^6 spleen cells per ml. Cells were then washed twice in medium and infectious centres were assayed *in ovo* according to the method described previously⁹. For determination of infectious centres after *in vivo* exposure to MDV, groups of five Hy-line SC (B^2/B^2) third-party chicks were given 500R of γ -rays on days 3 and 7 post-hatching and reconstituted with 1×10^8 spleen cells obtained from 6-week-old line-6 or line-7 donors. Following a 7-d period of natural exposure of the recipients to MDV, spleens from each group were pooled and the number of infectious centres was assayed *in ovo*⁹ in B^2/B^2 eggs histocompatible with the donor MHC. Background host-specific infectious centres in unreconstituted, γ -irradiated, MDV-exposed chicks formed less than 15% of the observed totals and this value was subtracted from the data to obtain donor-specific infectious centres. Error values are 1 s.d. from the mean.

to postulate that non-MHC-associated resistance could be due to differential infectivity, transformability or proliferation of the target cell for MDV. Susceptible line-7 birds display a much higher leukocyte-associated titre of MDV than resistant line-6 birds following natural exposure to MDV⁷. Although this observation is consistent with the target-cell hypothesis, it is explained equally well by immune surveillance mechanisms. However, differential infectivity is also demonstrable in line-6 and line-7 embryos⁹ at stages of development during which immune mechanisms are unlikely to be involved. This finding supports the target cell hypothesis rather than classical immunological surveillance mechanisms. Chickens which are genetically resistant to MDV nevertheless become infected with and shed virulent MDV. Furthermore our own evidence shows that embryonic fibroblasts from lines 6 and 7 are equally permissive to *in vitro* infection with a related herpesvirus, the herpesvirus of turkeys (HVT, Fig. 1). Similar observations have been made for MDV infection of adult kidney cells. However, one cannot assume from this that genetic resistance to lymphoma development could not be due to differential infectivity at the level of the target cell. Productive infection of fibroblasts or epithelial cells by MDV, a process which may reflect a capacity for infection at the level of the whole animal, is not equivalent to infection and neoplastic transformation of T lymphocytes and, thus, may not be subject to the same genetic restrictions. To determine whether line-6 and line-7 lymphocytes display differences in viral adsorption or infectivity demonstrable *in vitro*, spleen cells from these lines were incubated with HVT, pelleted by centrifugation, and the amount of infectious virus remaining in the supernatant was assayed on chick embryo fibroblast monolayers. HVT was used in these studies because it is much more stable in cell-free form than MDV, but it may be assumed that these two very similar herpesviruses use the same cellular receptors.

Spleen cells from line-7 (susceptible) birds adsorbed HVT in a dose-dependent fashion but line-6 spleen cells adsorbed less virus at the concentrations tested (Fig. 2). This difference is highly reproducible: this experiment has been repeated six times with the same result. In parallel experiments not presented here, line-7 thymocytes also adsorbed significantly more HVT than did line-6 thymocytes¹⁰. However, the difference in adsorptive capacity of spleen cells was greater than that observed for thymocytes, as might be expected if a mature T lymphocyte were the target for MDV.

Table 2 Marek's disease mortality in chickens which received thymus transplants

	Group				
	A	B	C	D	E
	Line 7 N→Line 6 TX	Line 7 γR→Line 6 TX	Line 6 N→Line 6 TX	Line 6 TX	Line 6 Sham TX
Total	38/72	10/24	6/36	12/46	3/17
%	52.8	41.7	16.7	26.0	17.6

Newly hatched chicks were thymectomised or sham-operated as described previously¹⁹. For thymus transplants, thymus lobes were cut into two or three pieces and immediately transplanted subcutaneously lateral to the oesophagus in areas which had been vacated by the host's thymus lobes. At least one entire thymus equivalent (14 lobes) was transplanted per recipient. On day 2 post-hatching chicks were contact exposed to MDV by housing them next to 4–6-week-old chickens which had been inoculated with 10,000 *in ovo* lesion-forming units⁹ of the BC-1 isolate of MDV²⁰. The incidence of MD-specific deaths was recorded for 14 weeks at the end of which significant losses due to MD had ceased. N = normal, non-irradiated donor thymus; TX = thymectomised; γR = 1,000R γ-ray irradiated donor thymus. Using Fischer's calculation of exact probability, $P = 0.00046$ for a two-tailed comparison of groups A and C and $P = 0.033$ for a one-tailed comparison of groups B and C.

Following viral adsorption, the number of infected cells of each type was estimated after the cells had been washed and incubated for 24 h. Line-7 spleen cells induced 6.7 times more infectious centres than did the line-6 spleen cells (Table 1). To confirm this observation for MDV *in vivo*, line-6 and line-7 spleen cells were adoptively transferred into separate groups of irradiated, histocompatible, third-party recipients which were then naturally exposed to MDV. Following this procedure, over four times more line-7 spleen cells than line-6 spleen cells were infected with MDV (Table 1).

These experiments suggest that it might be possible to make a resistant (line-6) bird more susceptible to MD by providing a source of susceptible (line-7) target cells. For this purpose, two sources of line-7 cells were used for transplantation into line-6 recipients. First, we prepared classical haematopoietic chimaeras following the intravenous injection of line-7 embryonic spleen cells into line-6 embryos and found that this procedure did not result in a significant increase in MD mortality¹⁰. Thus, the mere presence of line-7 haematopoietic cells apparently did not result in an increased mortality due to MD. To provide a more direct source of T lymphocytes, the putative target cells for transformation, we transplanted line-7 thymuses into newly hatched, thymectomised line-6 recipients. The pooled data from six experiments (Table 2) show that this procedure rendered the line-6 birds much more susceptible to MD. We observed no

significant differences in MD mortality between thymectomised line-6 birds, sham-thymectomised line-6 birds and thymectomised line-6 birds which received a line-6 thymus transplant. This finding serves to control for the possibility that the line-7 → line-6 transplanted birds showed a higher mortality simply because the donor thymus failed to reconstitute a depleted T-cell compartment. In addition, there have been no reports that thymectomy *per se* has an effect on MD mortality, although neonatal thymectomy combined with irradiation¹¹ or anti-T cell serum treatment¹² can affect MD mortality and virus titre.

The finding that transplantation of susceptible thymuses made the resistant birds more susceptible but that the presence of haematopoietic cells from the susceptible line did not, could mean that (1) thymus transplants simply provide more susceptible target cells at the appropriate stage of differentiation than a more primitive population of embryonic spleen cells, or (2) the genotype of the thymic micro-environment may influence the target-cell phenotype, as stem cells can be induced to undergo differentiation into mature T cells in the thymus gland. In support of the latter possibility, we have found that transplanted, irradiated (1,000R) line-7 thymuses increased the susceptibility of line-6 chicks (Table 2). Further experiments will be necessary to determine whether line-6 stem cells undergo a phenotypic change under the influence of line-7 thymus glands. One prediction would be that unlike the adoptive transfer of embryonic spleen cells, adoptive transfer of line-7 adult spleen cells, a portion of which have presumably migrated through a susceptible thymic microenvironment, should be sufficient to increase susceptibility to MD. These experiments are in progress.

Thus, we have presented data which strongly suggest that genetic resistance to an oncogenic herpesvirus can be manifested at the level of the target cell. Genetic resistance to C-type RNA viruses at the cell level has been shown in several systems^{13–16} but we believe that our studies represent the first demonstration of cellular resistance to an animal DNA virus. We transplanted susceptible thymuses into resistant animals and made them much more susceptible to oncogenesis. Conversely, Shieh and Sevoian¹² reported no significant effect of transplantation of a susceptible thymus into a thymectomised resistant host in lines of chickens in which resistance was associated with the MHC. Our data provide strong evidence that the target cell for MDV-induced transformation is a T lymphocyte. The mechanism of resistance at the target T-lymphocyte level remains unknown but our viral adsorption studies suggest that resistant birds may have target cells with fewer virus receptors, receptors of lower affinity for virus, or fewer target cells with identical receptors. That differences may exist between line 6 and line 7 in the size of T-cell subpopulations is suggested by other studies which show that line-7 birds possess three times as many alloreactive T cells as line-6 birds¹⁷ and that susceptible T cells may proliferate at a greater rate than resistant T cells¹⁸. In view of this, this, the observed differences in MDV infectious centres assayed *in ovo* are indicative of target cell phenotype, but may reflect the capacity of inoculated cells to divide and/or

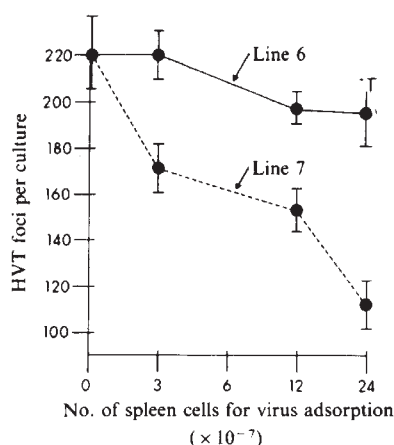


Fig. 2 HVT was prepared as described in Fig. 1. Cell suspensions from spleens of 3–4-week-old donors were prepared by collagenase digestion, washed three times in medium and mixed with aliquots of HVT. Only those cells having morphologies characteristic of lymphocytes were counted and included in the calculation of spleen cell number for data shown in Fig. 2 and Table 1. Virus adsorption was carried out at room temperature for 1 h and the cells were then pelleted by centrifugation. The amount of infectious HVT remaining in the supernatant was estimated by focus formation on replicate cultures of CEF of Hy-line SC origin. Error bars indicate 1 s.d. from the mean.

spread in the embryo. A role for such post-adsorptive mechanisms can only be ruled out by the careful use of serological and physical cell markers.

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- Epstein, M. A. in *Oncogenesis and Herpesviruses* (eds Biggs, P. M. et al.) 261–274 (IARC Publ. No. 2, 1972).
- de-Thé, H. in *Oncogenesis and Herpesviruses* (eds Biggs, P. M. et al.) 275–284 (IARC Publ. No. 2, 1972).
- Nazerian, K. in *Oncogenesis and Herpesviruses* (eds Biggs, P. M. et al.) 59–73 (IARC Publ. No. 2, 1972).
- de-Thé, G. et al. *Nature* **274**, 756–761 (1978).
- Longenecker, B. M., Pazderka, F., Gavora, J. S., Spencer, J. S. & Ruth, R. F. *Immunogenetics* **3**, 401–407 (1976).
- Briles, W. E., Stone, H. A. & Cole, R. K. *Science* **195**, 193–196 (1977).
- Fredericksen, T. L., Longenecker, B. M., Pazderka, F., Gilmour, D. & Ruth, R. F. *Immunogenetics* **5**, 535–552 (1977).
- Powell, P. C., Payne, L. N., Frazier, J. A. & Rennie, M. in *Oncogenesis and Herpesviruses II* Part 2 (eds de-Thé, G. et al.) 89–100 (IARC Publ. No. 11, 1975).
- Longenecker, B. M., Pazderka, F., Stone, H. S., Gavora, J. S. & Ruth, R. F. *Infect. Immun.* **11**, 922–931 (1975).
- Gallatin, W. M. & Longenecker, B. M. *Proc. EEC Symp. Resistance and Immunity to Marek's Disease*, Berlin (in the press).
- Sharma, J. M., Witter, R. L. & Purchase, H. G. *Nature* **253**, 477–479 (1975).
- Shieh, H. K. & Sevoian, M. *Comparative Leukemia Research 1975* (eds Clemmensen, J. & Yohn, D. S.) 396–399 (Bibl. Haemat. No. 43, 1976).
- Odaka, T. *J. Virol.* **3**, 543–548 (1969).
- Payne, L. N., Pani, P. K. & Weiss, R. A. *J. gen. Virol.* **13**, 455–462 (1971).
- Purchase, H. G., Gilmour, D. G., Romero, C. H. & Okazaki, W. *Nature* **270**, 61–62 (1977).
- Vogt, P. K. & Ishizaki, R. *Virology* **30**, 664–672 (1965).
- Pazderka, F., Longenecker, B. M., Law, G. R. J., Stone, H. A. & Ruth, R. F. *Immunogenetics* **2**, 93–100 (1975).
- Longenecker, B. M., Pazderka, F. & Ruth, R. F. *J. Immunogenet.* **2**, 59–67 (1975).
- Longenecker, B. M., Brietenbach, R. P. & Farmer, J. N. *J. Immun.* **97**, 594–599 (1966).
- Spencer, J. L., Gavora, J. S., Grunder, A. A., Robertson, A. & Speckman, G. A. *Avian Dis.* **18**, 33–44 (1974).

Feedback inhibition by human primed T-helper cells

STUDIES in animal models, in particular that of the murine system, have revealed that the sequence of events leading to an antibody response is similar to other complex biological processes in that they are controlled by interrelated regulatory mechanisms^{1,2}. In man helper activity which regulates the antigen-induced antibody response *in vitro* is initiated by T lymphocytes bearing receptors for the Fc fragment of IgM ($T\mu^+$ cells), whereas suppressive activity can be induced in T cells capable of binding the Fc fragment of IgG ($T\gamma^+$ cells)³. We present here data which indicate that $T\mu^+$ cells, apart from providing help in the human antigen-driven process of B-cell activation, are also capable, in certain conditions, of inducing a subpopulation of unprimed T cells to suppress an antigen-induced antibody response.

As has been reported⁴, the *in vitro* induction of suppressor activity by $T\gamma^+$ cells requires the presence of a relatively high dose of antigen in the culture, whereas helper activity of $T\mu^+$ cells prevails over suppressor activity if a relatively low dose of antigen is applied to a culture of unselected human peripheral T and B cells⁵. In an attempt to induce and measure helper activity without simultaneous activation of $T\gamma^+$ cells, $T\mu^+$ cells were isolated from the unselected T cells, added to purified B cells and stimulated by a dose of antigen known to induce a suppressor activity in cultures containing unselected T cells.

As can be seen in Table 1 (and ref. 3), considerable numbers of plaque-forming cells (PFC) were obtained, indicating that effective T-helper activity is generated if a high dose of antigen is used. Moreover, when isolated $T\mu^+$ cells were primed by pre-

culturing the cells with a high dose of sheep erythrocytes (SE) for 6 days and subsequently added to purified B cells, a PFC response is obtained that exceeds the number of PFC of B-cell cultures to which only unprimed $T\mu^+$ cells were added (see Table 1). When, however, primed $T\mu^+$ lymphocytes were added to a suspension of B cells supplemented with unselected, unprimed T cells, a low PFC response was observed, indicating that a negative regulatory activity had been generated by the subsequent addition of unselected, unprimed T cells. Figure 1 also shows that as the number of primed T-helper cells, added to a culture of unselected, unprimed T cells and B cells, increases, suppression also increases; this meets the definition of a feedback type of suppression.

Shore *et al.*⁶, who induced optimal helper activity by using 24-h primed $T\mu^+$ cells, did not observe any sign of a feedback inhibition of the antibody response when the cells were cultured with unprimed T cells and B cells. On the contrary, these cells reacted as adequate primed T-helper cells.

Our results lead us to suggest that during the 6 × 24 h priming period some differentiation and/or education of $T\mu^+$ cells occurred which resulted in their capacity to suppress the B-cell response. However, our data do not allow us to state definitely that a feedback signal is provided by $T\mu^+$ helper cells as such. Within the $T\mu^+$ subset, cells could be present which do not express antigen-specific help, but which are programmed exclusively to induce a feedback type of suppression. Cantor *et al.*⁷, however, when describing a similar system for the mouse, reported that the primed cell which is capable of delivering the feedback signal in the murine system, is a cell residing in the $Lyt\ 1^+$, $Qa\ 1^+$ subpopulation. It appears that the $Lyt\ 1^+$, $Qa\ 1^+$ cell is a T-helper cell capable of synergising with a $Lyt\ 1^+$, $Qa\ 1^-$ T cell to generate optimal helper activity. We cannot yet substantiate our hypothesis further because we are unable to visualise membrane structures that will allow the recognition of possible subsets of $T\mu^+$ cells.

Table 1 Regulation by SE-primed T helper cells

Primed T cells	Assay system*	PFC per 10^6 Lyt
None	+ 3.5 $T\mu^+$	2,333 (1,514–2,922)
3.5 $T\mu^+$	None	3,804 (2,581–4,201)
1.0 $T\mu^+$	+ 2.5 T^+	178 (0–364)
None	+ 2.5 T^+ + 1.0 $T\mu^+$	1,875 (900–2,224)

* Assay system: 1.5 B cells plus 5.0 SE (cell numbers × 10^6) plus indicated T-cell fractions.

† Unselected, unprimed T cells.

For the induction of PFC to SE, cultures were set up in 17 × 100 mm tissue culture tubes (Falcon) containing 1.5×10^6 B cells in 10 ml culture medium consisting of RPMI-1640, 25 μ g Fungizone, 100 μ g penicillin, 100 IU streptomycin, 2-mercaptoethanol (0.02 M), L-glutamine (2 mM) and supplemented with 10% serum derived from AB⁺ donors (AB serum) which had been preabsorbed with SE to prevent artefactual plaque formation¹¹. To these cultures various T-cell fractions were added: unprimed, unselected T cells (T^+) and/or $T\mu^+$ lymphocytes, together with 5×10^6 SE as antigen. Cultures were kept in a humidified atmosphere of 5% CO_2 in air. After culturing the cells for 6 d, cells were collected, washed twice in RPMI-1640 at 4°C and assayed for PFC according to Dosch and Gelfand¹². The data shown are expressed as the arithmetical mean of values obtained in five individual experiments. Within each experiment determinations were done in sixfold. Values in parentheses represent the ranges. *Priming procedures*: 15×10^6 $T\mu^+$ cells, which were isolated as described in Table 2 legend, were cultured with 15×10^7 SE in the inner tube of a Marbrook k-Diener type of culture tube. The inner tube contained 3 ml of culture medium supplemented with 15% SE-absorbed AB serum. The outer tube contained the same medium as the inner tube with the exception that the medium was supplemented with 5% fetal calf serum instead of 15% AB serum. The Marbrook-Diener cultures were kept for 6 d in a humidified atmosphere of 5% CO_2 in air. After the culture period the primed cells were freed of the SE by NH_4Cl -induced lysis. The cells were then washed twice with RPMI-1640 containing 5% AB serum. Viability of the cells was tested for by Trypan Blue dye exclusion which always exceeded 90%.

Table 2 Unprimed T-cell subsets mediating feedback inhibition

Primed $T\mu^+$ cells	Assay system*	PFC per 10^6 Lyt
3.5	+None	4,857
1.0	+2.5 T^+	186
1.0	+2.5 $T\mu^+$	3,414
1.0	+2.0 $T\mu^+$ + 0.5 T^+	364
1.0	+2.0 $T\mu^+$ + 0.5 $T\mu^+$	3,414
1.0	+2.0 $T\mu^+$ + 0.5 $T\gamma^+$	2,167
1.0	+2.0 $T\mu^+$ + 0.5 $T\mu, \gamma^-$	283
None	+3.5 $T\mu^+$	2,333
None	+3.0 $T\mu^+$ + 0.5 T^+	2,175
None	+2.5 $T\mu^+$ + 1.0 T^+	2,202
None	+2.0 $T\mu^+$ + 1.5 T^+	2,257

PBL, isolated according to Bøyum¹³, were separated into T and non-T cells by two cycles of E-rosetting and subsequent density gradient centrifugation on Ficoll-Isopaque ($\rho = 1.077$).³ The non-T fraction was depleted of non-lymphoid cells by adherence to plastic and recovered by gently scraping with a rubber policeman as described in ref. 3. $T\mu^+$ cell isolation: The T cell fraction was incubated with 3 mM theophylline (Sigma) for 60 min at 37°C (ref. 14). Cells were then collected by centrifugation at 4°C and immediately mixed with a 50-fold number of SE¹⁵. This mixture was layered on Ficoll-Isopaque ($\rho = 1.077$) and centrifuged for 30 min at 1,000 g. After the separation procedure the theophylline-resistant cells in the pellet were freed of contaminating SE by lysis with NH_4Cl and tested for purity. It was found that the above mentioned procedure resulted in a population of 95–99% $T\mu^+$ cells. $T\gamma^+$ and $T\mu, \gamma^-$ isolation: The interphase fraction of the above mentioned gradient, consisting of the theophylline-sensitive cells, was rosetted with ox erythrocytes coated with rabbit anti-ox IgG¹⁶. The cell mixture was pelleted and incubated on ice for 20 min. Then the pellet was gently resuspended and layered on Ficoll-Isopaque ($\rho = 1.077$). After centrifugation (30 min, 1,000 g) the pellet was freed of contaminating ox erythrocytes by NH_4Cl -induced lysis. The interphase (mainly consisting of $T\mu, \gamma^-$ cells) and the pellet fractions were tested for purity by determination of the number of $T\mu^+$ and $T\gamma^+$ cells present in the various fractions. Immediately after separation the pellet fraction contained 99% $T\gamma^+$ cells. After culturing overnight the Fc γ R could no longer be detected (ref. 3). Primed $T\mu^+$ cells were cultured with B cells, SE and when mentioned, with the various T-cell fractions. One representative experiment out of five is given.

* Assay system: 1.5 B cells + 5 SE (cell numbers $\times 10^6$). † Unprimed, unselected, autologous T cells.

To characterise further the cell type receiving the signal from the primed $T\mu^+$ cells, we separated the unprimed, unselected T-cell suspension into $T\mu^+$, $T\gamma^+$, and T cells without detectable Fc receptors for IgM and IgG (therefore referred to as $T\mu, \gamma^-$ cells), by means of a number of isolation procedures (see Table 2 legend). We tested the cells for their potential inhibitory capacity by adding the different subsets separately to cultures of primed $T\mu^+$ cells and B cells, supplemented with a low dose of antigen. It is clear from the results listed in Table 2 that: (1) unprimed $T\mu^+$ cells apparently do not receive the signal provided by the primed $T\mu^+$ cells; (2) unprimed $T\gamma^+$ cells which can suppress the response when they are cultured with a high dose of antigen, do not suppress the response in this system when they are co-cultured with primed $T\mu^+$ cells and a low dose of antigen; (3) unprimed $T\mu, \gamma^-$ cells are capable of interacting with the primed $T\mu^+$ cells, resulting in an inhibition of the PFC response (results of five independent experiments). It is postulated that unprimed $T\mu, \gamma^-$ cells are capable of recognising the stage and intensity of the immune response by detecting or receiving signal(s) from primed T-helper cells, which can result in a feedback mechanism and thereby regulate the response.

The activity of the supernatants of the primed $T\mu^+$ cell cultures was examined for their capacity to induce feedback inhibition of the PFC response *in vitro* because of an increasing awareness of the fact that many T-T interactions do not seem to require contact of the relevant cells, but are mediated by soluble factors released from the participating cells^{8,9}. As can be seen in Table 3, no inhibition of the PFC response could be obtained by the use of supernatants of primed $T\mu^+$ cells. On the contrary,

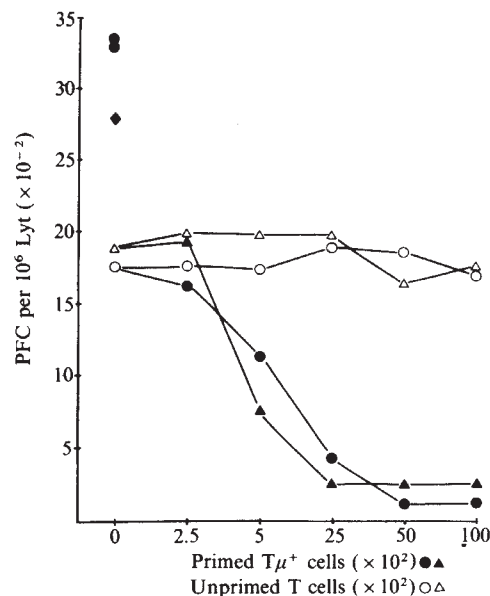


Fig. 1 Different numbers of primed $T\mu^+$ cells were added to a fixed number (3.5×10^6) of unprimed, unselected T cells (▲, ●; two donors). As a control the same numbers of unprimed, unselected T cells, derived from the two donors, were added to the same fixed number of unprimed, unselected T cells (△, ○). ◆, ● represent the PFC responses when only primed $T\mu^+$ cells from the two donors were cultured with purified B cells. Every culture contained a low dose (5×10^6) of SE.

only a potentiating (helper) effect of the T-cell supernatant was observed.

We do not yet know whether this type of T-T interaction plays any physiological role in the regulation of the *in vivo* immune response in man. Cantor *et al.*¹⁰ reported the absence of feedback suppression and a concomitant defect in the differentiation and/or function of T cells expressing the Lyt 123⁺ phenotype in NZB mice. It was suggested¹⁰ that the evidence provided by *in vitro* and *in vivo* experiments indicates that the absence of functional Lyt 123⁺ cells and consequent lack of feedback suppression are somehow related to the development of autoimmune reactivity in NZB mice.

For the present we accept as a working hypothesis that a subset of T lymphocytes characterised as $T\mu, \gamma^-$ can be regarded as the human analogue of the murine Lyt 123⁺ cell in the loop of a feedback inhibition mechanism of T-helper function. Therefore, we are investigating T-cell-mediated feedback suppression of the antigen-induced antibody response of peripheral leukocytes of patients suffering from systemic lupus erythematosus and myasthenia gravis. This investigation may yield information concerning the biological and clinical relevance of feedback inhibition of T-helper function in man. Moreover, it may to

Table 3 The activity of supernatants of primed $T\mu^+$ cell cultures

Primed $T\mu^+$ cells or supernatant	Assay system*	PFC per 10^6 Lyt
25% Supernatant $T\mu^+$	+None	4,935 (PFC per B Lyt)
3.5 $T\mu^+$	+None	3,601
25% Supernatant $T\mu^+$	+2.5 $T\mu^+$ + 1 T^+	2,670
1 $T\mu^+$	+1.5 $T\mu^+$ + 1 T^+	232
None	+2.5 $T\mu^+$ + 1 T^+	2,275

The supernatant of the primed $T\mu^+$ cell culture in the inner tube was collected after 6 d and centrifuged for 10 min at 2,300g. After Millipore filtration (0.22 μ m) the supernatant was immediately added to the cultures as listed in Table 3.

* Assay system: 1.5 B cells plus 5.0 SE (cell numbers $\times 10^6$) plus indicated T-cell fractions. † Unselected, unprimed T cells.

some extent explain the dysregulation of T-cell-dependent antibody responses which may have a role in the aetiopathogenesis of these autoimmune diseases.

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1. Eardley, D. D. *et al.* *J. exp. Med.* **147**, 1106–1115 (1978).
2. McDougal, J. S., Shen, F. W. & Elster, P. J. *Immun.* **122**, 437–442 (1979).
3. Heijnen, C. J., UytdeHaag, F., Gmelig-Meyling, F. H. J. & Ballieux, R. E. *Cell. Immun.* **43**, 282–292 (1979).
4. UytdeHaag, F., Heijnen, C. J. & Ballieux, R. E. *Nature* **271**, 556–557 (1978).
5. Heijnen, C. J., UytdeHaag, F., Dollekamp, I. & Ballieux, R. E. in *Antibody Production in Man: in vitro Synthesis and Clinical Implications* (eds Fauci, A. S. & Ballieux, R. E.) (Academic, New York, in the press).
6. Shore, A., Dosch, H. M. & Gelfand, E. W. *Nature* **274**, 586–587 (1978).
7. Cantor, H. *et al.* *J. exp. Med.* **148**, 871–877 (1978).
8. Kantor, F. S. & Feldmann, M. *Clin. exp. Immun.* (in the press).
9. Heijnen, C. J., UytdeHaag, F. & Ballieux, R. E. in *Proc. 12th Leucocyte Culture Conference: Biochem. and Cell Biol. of Leucocyte Function* (ed. Quastel, M. R.) (in the press).
10. Cantor, H. *et al.* *J. exp. Med.* **147**, 1116–1125 (1978).
11. Muchmore, A. V., Koski, J., Dooley, N. & Blaese, R. M. *J. Immun.* **116**, 1016–1019 (1976).
12. Dosch, H. M. & Gelfand, E. W. *J. Immun.* **118**, 302–308 (1977).
13. Bøym, A. *Scand. J. clin. lab. Invest.* **21**, Suppl. 97 (1968).
14. Limatibul, S., Shore, A., Dosch, H. M. & Gelfand, E. W. *Clin. exp. Immun.* **33**, 503–513 (1978).
15. Gmelig-Meyling, F. & Ballieux, R. E. *Vox Sang.* **33**, 5–8 (1977).
16. Moretta, L., Webb, S. R., Grossi, C. E., Lydyard, P. M. & Cooper, M. D. *J. exp. Med.* **146**, 184–200 (1977).

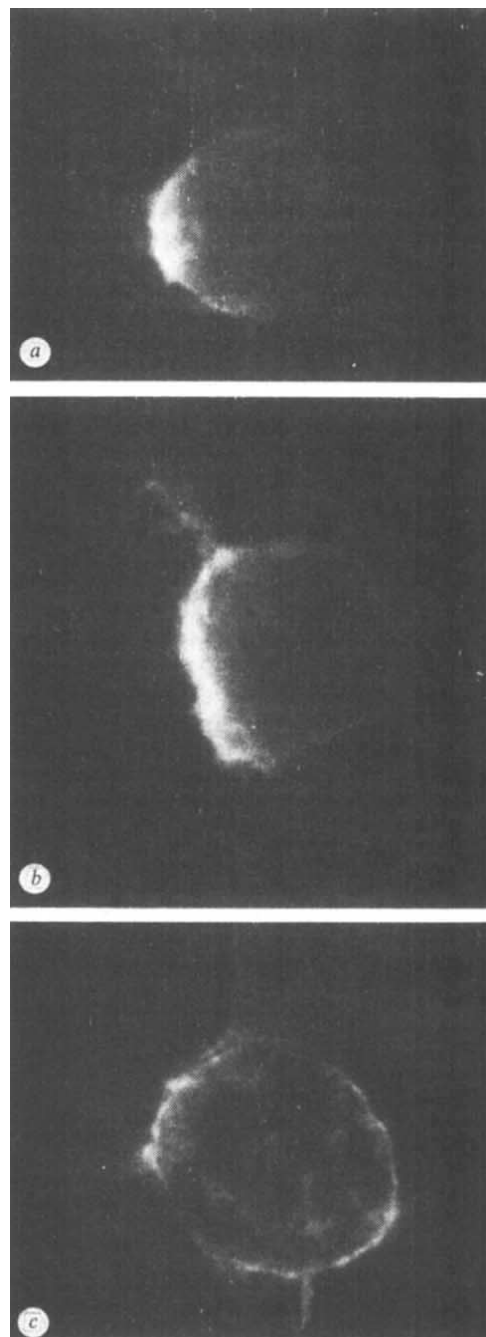


Fig. 1 TRITC-Con A-labelled NS-20 cells in a steady electric field observed by fluorescence microscopy. Conditions for a steady electric field were similar to those described by Poo *et al.*⁸, Ag–AgCl electrodes, agar bridges and culture chamber made from microscope slide and pieces of no. 1 coverslips. After finding the 'stationary conditions' we used a uniform electric field of 12 V cm^{-1} for 15 min at room temperature ($19 \pm 2^\circ\text{C}$) in PBS. The cells ($\sim 100,000$ cells for the chamber) were grown for 1 day in the chamber with F10 medium for the C_6 and with minimum essential medium for the NS-20 and 3T3 lines. All media were supplemented with 10% fetal serum and antibiotics. The conjugates TRITC (Merieux) with Con A (Pharmacia) and ricin (molecular weight 120,000) were attached to Sephadex G-200 or Sepharose 4B, respectively, with subsequent elution of the sugars and passage through biobel P30. The cells were labelled with TRITC-labelled lectins at a concentration of $150 \mu\text{g ml}^{-1}$ for 10 min at $0-4^\circ\text{C}$ before or after electrophoresis, and fixed with 2% paraformaldehyde ($0-4^\circ\text{C}$ for 10 min). Cells rapidly fixed with cold acetone ($0-4^\circ\text{C}$, 30 s) had a similar distribution of receptors. *a*, *b*, The cells were exposed to an electric field before Con A-TRITC labelling and either fixed immediately (*a*) or were allowed to remain in MEM medium at room temperature for 35 min before fixation (*b*). *c*, The cells were exposed to a field after Con A-TRITC labelling. The photographs were taken using a Zeiss microscope equipped with Opak-Fluor vertical illumination. Control cells not exposed to the electric field showed uniform ring staining ($\times 2,124$).

Does lectin–receptor complex formation produce zones of restricted mobility within the membrane?

It is well established that component lipids and proteins are mobile in the plane of the membrane^{1,2}. This lateral mobility could have an important role in the transmission of signals through the membrane. For example, there is much evidence that aggregation of receptors induced by divalent antibodies or by chemical cross-linking can reproduce the biological action of the corresponding ligands^{3–5}. It was shown recently that receptors for monovalent ligands like insulin and epidermal growth factor aggregate into patches on the cell surface after interaction with the corresponding hormone. Clustered receptors accumulate at coated regions of the membrane and become internalised in endocytic vesicles^{6,7}. Aggregation is dependent on receptor occupancy by the active ligand⁷. Here we show that lateral mobility is also dependent on receptor occupancy. Our approach has been to compare the lateral mobilities of free and ligand-bound receptors using cultured cells placed in a steady electric field. This permits the measurement of lateral long-range (over the whole cell) mobility of the receptors with and

Table 1 Values of the diffusion constant (D) obtained by the electrophoretic (without ligand) and FPR methods

Cells	Electrophoresis ('free receptor')		FPR (ligand-receptor complex)			
	Con A D	Ricin D	Con A* D	%R D	Ricin D	%R D
NS-20	$2-3 \times 10^{-10}$	$2-3 \times 10^{-10}$	$6.0 \pm 0.5 \times 10^{-11}$	30% (20-50%)	$5.5 \pm 0.5 \times 10^{-11}$	~30%
C ₆	$2-3 \times 10^{-10}$	2×10^{-10}			$6.0 \pm 0.5 \times 10^{-11}$	~40%
3T3	$1-2 \times 10^{-10}$					

In the photobleaching experiments carried out on dispersed Con A-receptor complexes, a Spectra-physic argon ion laser operating at <10 mW at 514 nm was used to bleach the spot and the same attenuated beam to excite fluorescence in the spot laser beam was passed into a Leitz microscope^{9,10}. The laser beam diameter (3.4 μ m) and the D values were determined with the calibration scan described by Axelrod *et al.*¹². Coefficients expressed in $\text{cm}^2 \text{s}^{-1}$; temperature of measurements, $19 \pm 2^\circ \text{C}$; %R, the per cent of the fractional fluorescence recovery.

*Also published values of Y.A.Z. *et al.*¹⁰ obtained by the 'initial slope' calculations.

without the corresponding ligands⁸. We have compared this method with the dynamic method of fluorescence photobleaching recovery (FPR) which only permits measurement of the mobility of fluorescent ligand-receptor complexes in the range of the size of the laser beam diameter ($\sim 3 \mu\text{m}$)⁹⁻¹². We have studied the lateral mobility of concanavalin A (Con A) and ricin receptors from non-malignant fibroblasts 3T3 and malignant neuroblastoma NS-20 and C₆ glial cells with both techniques. Our results suggest that lectin-receptor formation results in the production of zones of restricted mobility in the membrane.

Results obtained by the 'electrophoretic' technique are shown in Figs 1 and 2. Application of an electric field to the extracellular compartment produced a migration of free Con A receptors towards the cellular membrane facing the positive pole (Fig. 1a). Removal of the electric field resulted in a redistribution of receptors over the whole cell surface. Determination of the time course of the redistribution (Fig. 2) enabled the lateral diffusion constant, D , to be calculated, using the equation $D = r^2/2\tau$, where r is the cell diameter and τ is the $1/e$ relaxation time. Values of D obtained in the various experimental situations tested are listed in Table 1. For the free receptors, all values were in the range $1-3 \times 10^{-10} \text{ cm}^2 \text{s}^{-1}$. These values are close to that obtained using the FPR technique on the pool of surface membrane proteins accessible to nonspecific labelling by fluorescein isothiocyanate^{9,13}; they are also similar to values obtained for free Con A and surface antigen receptors^{8,14}. In agreement with the conclusions of Edidin and Wei¹⁴, the cytoskeletal elements do not seem to have any effect on the mobility of 'free' lectin receptors, as shown by experiments with drugs like cytochalasin B, colchicine and vinblastine. Finally, we note that the asymmetry observed after application of an electric field was less than 100% (Fig. 2). This reflects the fact that the cell population was heterogeneous with respect to the mobility of lectin receptors. For any given cell exhibiting receptor mobility the per cent asymmetry between the cellular borders facing the negative and positive poles was close to 100% (Fig. 1).

To measure the lateral mobility of ligand-bound receptors, free receptors were made to accumulate at one cell pole. The fluorescent lectins were then added immediately after removal of the electric field and the ligand-bound receptors were allowed to diffuse for various time intervals before the cells were fixed for microscopic examination. In all cases studied (Con A receptors from all cell lines used and ricin receptors from C₆ cells), no noticeable redistribution occurred within 35 min (Fig. 1b). We considered that this could mean the ligand-receptor complexes on the cells had become immobilised. To confirm this suggestion, the cells were treated with the ligand before electrophoresis, but there was no asymmetric redistribution of the receptors (Fig. 1c). This last result agrees with the observation of Poo *et al.*⁸ for Con A receptors of frog muscle cells. In contrast, the use of the FPR technique to measure the lateral mobility of the lectin-lectin receptor complexes gave strikingly different results. The apparent diffusion constant ($5-6 \times 10^{-11} \text{ cm}^2 \text{s}^{-1}$, Table 1) was only 5 times less than the diffusion constant of the free receptors as determined by the electrophoretic technique. Values of D did not change during at least 2 h of measurement at room temperature in phosphate-buffered saline (PBS).

We can resolve this apparent discrepancy by suggesting that after interaction with the ligands the mobility of the receptors becomes restricted in specific regions. To investigate this suggestion, we carried out successive bleachings of tetramethylrhodamine isothiocyanate (TRITC)-Con A complexes of the same region of the membrane of NS-20 cells (Fig. 3). If the receptors have complete mobility, we would expect (after the third bleaching) $\sim 90-100\%$ recovery of fluorescence (%R) (almost all the immobile molecules in the spot should be bleached with a bleaching parameter $K \approx 5$), but if receptor mobility is restricted fluorescence recovery should be substantially less than this. The results obtained (mean %R value after third bleaching $\approx 50\%$, nine experiments) support the suggestion that mobility of the receptors occurs in limited regions. Although the topology of the fluid stationary 'fields' in which these complexes move is unknown, the area of the fields seems to be of the order of the area of the laser beam ($\sim 10 \mu\text{m}^2$).

Cross-linking of receptors by multivalent lectins does not seem to be a major factor in the process of ligand-induced receptor immobilisation, because (1) receptor immobilisation could be induced using ricin R 60 (60,000 molecular weight form) which has only one binding site for sugar, and (2) the polyene antibiotic amphotericin B suppressed ligand-induced immobilisation of Con A and ricin receptors from C₆ and NS-20 cells¹⁵; amphotericin B did not affect lectin binding to receptors when added either before or after Con A or ricin and therefore cannot interfere with eventual receptor cross-linking.

We suggest that the appearance of these fields reflects a phase separation of membrane lipids which is triggered by ligand-receptor interaction¹⁶⁻¹⁸. There is evidence that a specifically

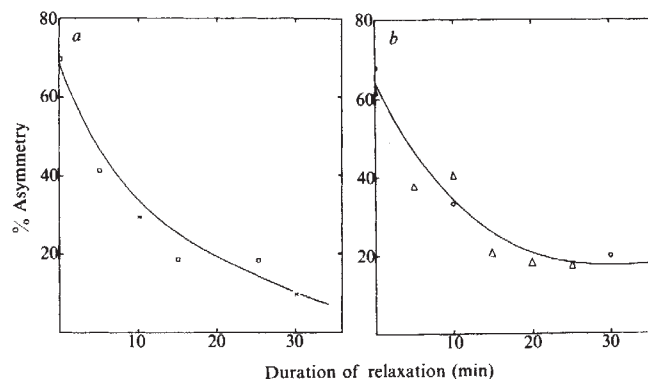


Fig. 2 Recovery of asymmetric distribution by relaxation. After removal of the field, the cells remained in the medium at room temperature for various periods of time before TRITC-lectin labelling and paraformaldehyde fixation. Per cent asymmetry = $(A - B/T)100\%$, where A and B are the number of cells showing asymmetric distribution of fluorescence towards the positive and negative poles, respectively, and T is the total number of cells examined. (Total number examined for each point was not less than 80.) In the case of long cells, we examined only cells which had their long axis aligned within 30° with respect to the direction perpendicular to the direction of the field. *a*, The relaxation of Con A receptors of NS-20 cells; the mean diameter of NS-20 cells was $14 \pm 0.5 \mu\text{m}$. *b*, The relaxation of Con A receptors of C₆ cells after treatment with colchicine, $5 \mu\text{g ml}^{-1}$ (O) and $1 \mu\text{g ml}^{-1}$ (x); the mean diameter of the C₆ cells was $16 \pm 0.5 \mu\text{m}$.

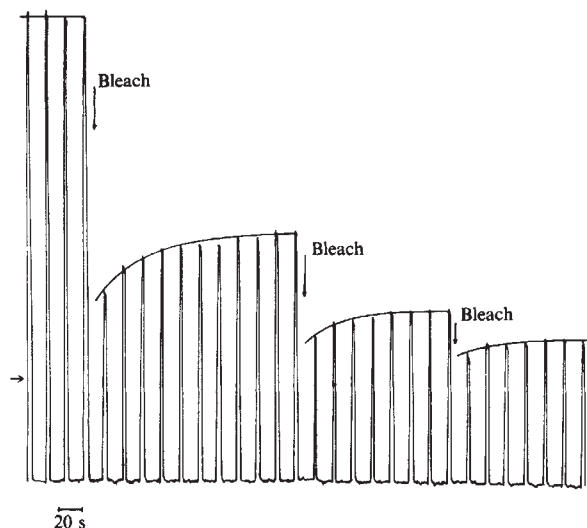


Fig. 3 Recorder trace of the fluorescence intensity of a successively bleached spot on TRITC-Con A-labelled NS-20 cells. Values for the recovery of fluorescence after the 1st, 2nd and 3rd bleachings were 21%, 40% and 43%, respectively. The modulation was made with an optic-acoustic modulator (SORO). The intensity of fluorescence below the horizontal arrow corresponds to light scattering.

immobilised boundary layer of lipid surrounds the integral membrane proteins. Interaction of a membrane protein with a specific ligand might modify its interaction with surrounding lipids through conformational changes¹⁸⁻²⁰, or, as recently suggested by Hirata *et al.*²¹ in the case of β -adrenergic receptors, through enzymatic conversion and catalysed 'flip flop' of membrane phospholipids in the vicinity of the receptor. In addition, changes in lipid fluidity after interaction of lectins with the membrane have been demonstrated²². A change in the lipid environment of lectin-lectin receptor complexes might facilitate their aggregation, leading to the occurrence of clusters surrounded by a more fluid zone of the membrane co-existing with the remaining pool of the membrane lipids and proteins. Our experimental results obtained with lectins at 20°C indicate that this state could correspond to a steady-state dynamic equilibrium.

We believe that the simplest way to explain this 'stationary phase' separation is to suggest a key role for cholesterol in the process. Cholesterol plays a critical part in creating a generally fluid phase, which would separate into liquid and gel phases if cholesterol were not present^{23,24}. Cholesterol has a preference for some lipids (for example, lecithin) and a tendency for self-aggregation, but has no affinity for proteins²⁴. Therefore, during microaggregation of receptors, cholesterol, which could have a preference for the new 'coat' lipids (like lecithin) of the receptors, could move from the 'common' membrane pool into the cavity created by the aggregated proteins, and the region external to the restriction zone could be the phase of the lipids with which the cholesterol has weak interactions (like phosphatidylethanolamine)²⁵ and which could be stabilised by Ca^{2+} ions²⁴. This could enable restricted zones of the membrane to be in equilibrium with the remaining membrane pool of lipids and proteins. Suppression of ligand-induced receptor immobilisation by amphotericin B, which specifically interacts with cholesterol (refs 15, 26) supports the proposed interpretation.

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1. Frye, L. D. & Edidin, M. *J. Cell Sci.* **7**, 319-335 (1970).
2. Singer, S. J. & Nicolson, G. L. *Science* **175**, 720-731 (1972).
3. Segal, D., Taurig, J. D. & Metzger, H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2993-2997 (1977).
4. Kahn, C. R., Baird, K. L., Jarrett, D. B. & Flier, J. S. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4209-4213 (1978).
5. Jacobs, S., Change, K. J. & Cuatrecasas, P. *Science* **200**, 1283-1284 (1978).
6. Maxfield, F., Schlessinger, J., Shechter, Y., Pastan, I. & Willingham, M. C. *Cell* **14**, 805-810 (1978).
7. Schlessinger, J. in *Physical Chemical Aspects of Cell Surface Events in Cellular Regulation* (eds Delisi, C. & Blumenthal, R.) (Elsevier, Amsterdam, 1979).
8. Poo, M. H., Poo, W. J., & Lam, J. W. *J. Cell. Biol.* **76**, 483-501 (1978).
9. Edidin, M., Zagayansky, Y. & Lardner, T. J. *Science* **191**, 466-468 (1976).
10. Zagayansky, Y., Benda, P. & Bisconte, J. C. *FEBS Lett.* **77**, 206-208 (1977).
11. Jakobson, K., Wu, E. & Poste, G. *Biochim. biophys. Acta* **433**, 215-222 (1976).
12. Axelrod, D., Koppel, D. E., Schlessinger, J., Elson, E. & Webb, W. W. *Biophys. J.* **16**, 1055-1069 (1977).
13. Schlessinger, J., Axelrod, D., Koppel, D. E., Webb, W. W. & Elson, E. L. *Science* **195**, 307-309 (1977).
14. Edidin, M. & Wei, T. J. *J. Cell Biol.* **75**, 475-482 (1977).
15. Zagayansky, Y. & Jard, S. *Cell* (submitted).
16. Trauble, H. & Overath, P. *Biochim. biophys. Acta* **307**, 491-512 (1973).
17. Jost, P. C., Griffith, O. H., Capaldi, P. & Vanderkoi, G. *Proc. natn. Acad. Sci. U.S.A.* **10**, 480-484 (1973).
18. Morch, O. & Barrantes, F. J. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4329-4333 (1978).
19. Beadling, L. & Rothfield, L. I. *Proc. natn. Acad. Sci. U.S.A.* **75**, 3669-3672 (1978).
20. Tsong, T. Y. & Yang, C. S. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5955-5959 (1978).
21. Hirata, F., Strittmatter, J. & Axelrod, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 368-372 (1979).
22. Toyoshima, S. & Osawa, T. *J. biol. Chem.* **250**, 1655-1660 (1975).
23. Oldfield, E. & Chapman, D. *FEBS Lett.* **23**, 285-297 (1972).
24. Wallach, O. F. H. *Membrane Molecular Biology of Neoplastic Cells* (Elsevier, Amsterdam, 1975).
25. Van Dijk, P. W. M., De Kruijff, B., Van Deenen, L. L. M., De Gier, J. & Demel, R. A. *Biochim. biophys. Acta* **455**, 576-587 (1976).
26. De Kruijff, B., Gerritsen, W. J., Oerlemans, A., Demel, R. A. & Van Deenen, L. L. M. *Biochim. biophys. Acta* **399**, 30-43 (1974).

(DTrp⁶-Pro⁹-NET)-luteinising hormone-releasing factor inhibits follicular development in hypophysectomised rats

THE super-luteinising hormone-releasing factors (super-LRFs) are a group of synthetic analogues at least 100 times more potent than the native peptide *in vitro* and *in vivo*¹. Several laboratories have demonstrated inhibitory effects of super-LRFs on ovulation, egg implantation, pregnancy, testicular weight and spermatogenesis²⁻⁶. Their proposed mechanisms of action involve some type of desensitisation of the pituitary to the highly potent LRF analogue and of the gonads to the gonadotropins released in large quantities by the super-LRF, in both target tissues through receptor down-regulation. Another, seemingly unlikely, explanation for the gonadal inhibitory effects of super-LRFs is that the synthetic peptide acts directly at the level of follicular ovarian tissues. Such a direct effect of super-LRF on the gonads has been suggested by the observation that large doses of LRF prevent cultured granulosa cells from aromatising androgens to oestrogens⁷. We now report that a super-LRF inhibits follicular development in hypophysectomised rats pretreated with pregnant mare serum gonadotropin.

Pregnant mare serum gonadotropin (PMSG) has predominantly FSH-like (follicular-stimulating hormone) activity and some LH-like (luteinising hormone) activity, and induces ovarian follicular development in intact as well as hypophysectomised immature rats⁸. Various doses of PMSG dissolved in 0.2 ml of 16% gelatin were given subcutaneously (s.c.) to 26-d-old female rats (from Hormone Assay, Chicago) approximately 24 h after hypophysectomy by the parathyroid route. Various amounts of the super-LRF, (DTrp⁶-Pro⁹-NET)-luteinising hormone-releasing factor (pGlu-His-Trp-Ser-Tyr-DTrp-Leu-Arg-Pro-N-ethylamide) dissolved in 0.1 ml physiological saline were given s.c. at 8 a.m. and 5 p.m. daily for 3 d. The ovarian weights were recorded on day 31 as the endpoint. In some groups 20 IU of HCG (human chorionic gonadotropin) in 0.25 ml of physiological saline was given

intraperitoneally at 1.30 p.m. on day 30 and the incidence of ovulation was determined as reported previously⁹. The sella turcica of each rat was examined under magnification to determine whether the entire pituitary had been removed. The few rats in which pituitary fragments were found were not included in the results. The multiple comparison tests of Dunnett and Duncan for significance of differences were used for statistical evaluation of the experiments after an analysis of variance (computer program EXBIOL).

Administration of various doses of PMSG in 16% gelatin, a vehicle which delays absorption, produced a dose-dependent increase in ovarian weight (Table 1). Injection of the super-LRF inhibited the ovarian follicular development induced by PMSG. In rats treated with 50 IU PMSG, the minimum effective dose of super-LRF to prevent follicular induction was 0.08 µg twice daily. A similar amount of native LRF or another analogue of LRF with D-amino acid substitutions [(D¹Phe²-D⁶Phe⁶)-LRF] given in the same manner did not inhibit follicular development in animals pretreated with 50 IU PMSG, suggesting that the inhibition was not due to chronic injection of any decapeptide with or without a D-amino acid substitution. Experiments were also carried out to determine the responsiveness of these PMSG-primed follicles to HCG in animals concomitantly injected with the super-LRF nonapeptide: none of the animals treated with super-LRF ovulated, whereas treatment with HCG-PMSG without super-LRF resulted in the well known super-ovulation (Table 2).

These results clearly show that an analogue of the hypothalamic LRF, the super-agonist (D¹Trp⁶-Pro⁹-NEt)-LRF, has a direct effect on the ovary *in vivo*, inhibiting the follicular maturation normally induced by large doses of PMSG. Hseuh and Erickson have reported *in vitro* inhibitory effects of large doses of LRF on the aromatisation of androgens to oestrogens⁷ by granulosa cells in monolayer cultures. Oestrogens are known to stimulate follicular maturation¹⁰; granulosa cells from medium-sized and small follicles do not grow or luteinise unless

Table 2 Inhibition of ovulation by super-LRF in hypophysectomised rats

Treatment			No. of rats ovulated total no. of rats	Average ovarian weight ± s.e. (mg)	Average no. of ova/per ovulating rat ± s.e.
PMSG (IU)	Super/LRF (µg)	HCG (µg)			
25	—	—	0/4	42.5 ± 2.4	—
25	20	—	0/3	5.7 ± 0.3*	—
25	—	20	4/4	56.1 ± 3.3‡	15.6 ± 0.8
25	20	20	0/6	6.7 ± 0.8*	—
50	—	—	0/4	75.7 ± 2.4	—
50	20	—	0/5	19.9 ± 0.9*	—
50	—	20	5/5	112.5 ± 6.0†	29.0 ± 0.4
50	20	20	0/5	21.1 ± 1.2*	—

Super-LRF (20 µg per 0.1 ml per injection) was given s.c. at 8 a.m. and 5 p.m. for 3 d.

they are first primed with both FSH and LH¹¹, but oestrogens seem to facilitate stimulation of the mitosis of granulosa cells which is primarily responsible for follicle growth¹⁰⁻¹². In the experiments reported here, no physiological responses to oestrogen secretion, such as extended uterine horns, increase in ovarian weight, and presence of proestrous vaginal smears and vaginal opening, were observed in super-LRF-treated rats whereas all these criteria were observed in the PMSG control animals. Thus, super-LRF itself seems to have adverse effects on steroidogenesis *in vivo* and consequently interferes with follicular development.

The inhibitory effect of super-LRFs has been suggested to be due to down-regulation of gonadotropin receptors in the gonads resulting from the extremely high level of gonadotropins induced by the super-LRFs^{6,14}. Regulation of gonadotropin receptors in the gonad is highly sensitive to the circulating gonadotropin concentration, a marked loss of receptor sites occurring after treatment with exogenous gonadotropins. Through such a negative regulation of gonadotropin receptors and desensitisation of gonadal responses, super-LRFs would adversely affect ovarian steroidogenic function⁶, ovulation⁴ and growth of endocrine-dependent mammary tumours¹⁵. Indeed, Catt *et al.* have reported that the gonadotropin receptor content of the gonad exhibited delayed and protracted changes after single doses of super-LRF¹⁴, and binding sites were totally abolished after treatment with 10 µg LRF. When the pituitary gland is removed as we report here, super-LRF must inhibit gonadal response to exogenous gonadotropin by acting directly at the gonadal level; this suggests that down-regulation of gonadotropin receptors may not be the only interpretation.

Judging from our observations that super-LRF directly inhibits follicular development, presumably due to interference with steroidogenesis, it is obvious that super-LRF may either bind to granulosa cells to alter the gonadotropin receptors or antagonise the subsequent secretory step of gonadotropin (PMSG) molecules by binding to the ovarian receptor site. Such a direct action of super-LRF on gonads and possibly on other reproductive organs, including the uterus, to explain its effects on egg implantation^{6,16}, would lead to the possibility of the local administration of super-LRF as a totally novel approach to contraceptive medication. Furthermore, we should not neglect the possibility that the inhibitory effects of super-LRFs on oestrogen-dependent mammary tumours could similarly be explained by or related to such a direct effect of the super-LRFs on the mammary tissues. Moreover, these observations and data reported by others¹¹⁻¹³ suggest that the gonad may contain and possibly secrete substances that might be included in a multi-step feedback system involving hypothalamic-pituitary peptides. Indeed, recent data from this laboratory have shown the presence in aqueous extracts from PMSG-stimulated ovaries, of both LRF-like and LRF-antagonistic activities, presumably of peptidic nature¹⁷.

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Table 1 Inhibition by super-LRF of follicular development in hypophysectomised rats

Treatment				n	Average ovarian weight ± s.e. (mg)
PMSG (IU)	Super-LRF (µg)	LRF (20 µg)	(D ¹ Phe ² -D ⁶ Phe ⁶)-LRF (20 µg)		
—	—	—	—	6	15.8 ± 0.9
12	—	—	—	6	16.8 ± 2.3‡
12	20	—	—	4	6.8 ± 0.6‡
12	1.2	—	—	3	6.2 ± 0.4*
12	0.3	—	—	4	8.0 ± 0.6*
12	0.08	—	—	3	8.2 ± 1.1†
12	0.02	—	—	5	16.5 ± 2.0‡
25	—	—	—	8	42.5 ± 2.4
25	20	—	—	7	7.1 ± 0.7*
25	5	—	—	4	8.5 ± 1.0*
25	1.2	—	—	4	10.3 ± 0.8*
25	0.3	—	—	4	17.4 ± 2.5*
25	0.08	—	—	4	36.4 ± 3.7‡
50	—	—	—	14	69.4 ± 5.7
50	—	—	+	5	83.1 ± 5.2‡
50	—	+	—	5	79.2 ± 7.9‡
50	20	—	—	12	13.6 ± 0.8*
50	5	—	—	5	7.0 ± 0.3*
50	1.2	—	—	5	10.4 ± 1.4*
50	0.3	—	—	7	24.4 ± 2.5*
50	0.08	—	—	8	49.7 ± 7.4*
100	—	—	—	4	93.5 ± 7.5
100	20	—	—	4	8.5 ± 1.6*
100	5	—	—	3	29.3 ± 2.0*
100	1.2	—	—	3	44.7 ± 2.7*
100	0.3	—	—	4	85.2 ± 11.8‡

Super-LRF [(D¹Trp⁶-Phe⁸)-LRF] at various doses, 20 µg LRF, or (D¹Phe²-Phe⁶)-LRF in 0.1 ml physiological saline were given s.c. at 8 a.m. and 5 p.m. for 3 d. Significance from PMSG control group: *P < 0.01; †P < 0.05; ‡ not significantly different (multiple comparison test of Dunnett). Applies also to Table 2.

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- Vale, W., Rivier, C. & Brown, M. A. *Rev. Physiol.* **39**, 473–527 (1977).
- Rippel, R. H. & Johnson, E. S. *Proc. Soc. exp. Biol. Med.* **152**, 432–436 (1976).
- Johnson, E. S., Gendrich, R. L. & White, W. F. *Fert. Steril.* **27**, 853–860 (1976).
- Corbin, A. et al. *Fert. Steril.* **28**, 471–475 (1977).
- Dutta, A. S., Furr, B. J. A., Giles, M. B., Valcassin, B. & Walpole, A. L. *Biochem. biophys. Res. Commun.* **81**, 382–390 (1978).
- Rivier, C., Rivier, J. & Vale, W. *Endocrinology* **103**, 2299–2305 (1978).
- Hseuh, A. J. W. & Erickson, G. F. *Science* **204**, 854–855 (1979).
- Ying, S. H. & Meyer, R. K. *Proc. Soc. exp. Biol. Med.* **139**, 1231–1233 (1972).
- Ying, S. H. & Meyer, R. K. *Proc. Soc. exp. Biol. Med.* **130**, 40–43 (1969).
- Bradbury, J. *Endocrinology* **68**, 115–120 (1961).
- Channing, C. P. *Endocrinology* **87**, 156–164 (1970).
- Thanki, K. H. & Channing, C. P. *Endocr. Res. Commun.* **3**, 319–333 (1976).
- Channing, C. P., Thanki, K. H. & Lindsey, A. M. in *Plasma Membranes, Hormone Action and Reproductive Biology* (ed. Birnbaumer, L.) (Plenum, New York, 1978).
- Catt, K. J., Bankal, A. J., Davies, T. F. & Dufau, M. L. *Endocrinology* **104**, 17–25 (1979).
- Danguy, A. et al. *Eur. J. Cancer* **13**, 1089–1094 (1977).
- Lin, Y. C. & Yoshinaga, K. *Proc. 58th Mtg Endocrine Soc.* **143**, abstr. (1976).
- Ying, S.-Y. & Guillemin, R. *Proc. XIth Int. Congr. Biochemistry* (in the press).

Endogenous opiates block dopamine inhibition of prolactin secretion *in vitro*

OPIATES stimulate prolactin secretion *in vivo*; plasma prolactin levels are increased by morphine administration in normal¹ and steroid-primed male rats², as well as in immature³ and oestrogen-treated female rats⁴. Met-enkephalin and β -endorphin were also stimulatory^{1–6} as were enkephalin analogues^{3,7,8}. Naloxone or naltrexone, specific opiate antagonists, reduce basal prolactin levels when given alone^{1,3,9}, and block, at least partially, the effect of opiate agonists^{1–4,7,8}. None of these drugs is active *in vitro* when tested alone on anterior pituitaries³ or dispersed pituitary cells^{2,4}. However, as we have previously shown in a preliminary experiment, the inhibitory effect of dopamine on prolactin secretion *in vitro* was antagonised by the addition of morphine to the incubation medium¹⁰. In the present work, we have attempted to clarify further the mode of interaction of opiates with prolactin secretion. We confirmed that morphine, Met-enkephalin and β -endorphin do not affect the spontaneous release of prolactin *in vitro*, but found that they suppress the inhibitory effect of dopamine on prolactin release. We have also characterised the specificity and the kinetics of this interaction.

To demonstrate the effect of these substances, paired control and experimental hemi-pituitaries of male rats (Wistar, Evic Seba) were incubated, under constant gassing (95% O₂, 5% CO₂), in medium 199 HEPES (Gypco) containing bacitracin (2×10^{-5} M) to prevent enzymatic degradation of peptides¹¹ and ascorbic acid (10^{-4} M) to protect dopamine against oxidation. Incubation time was 1 h following two 45-min preincubation periods. Both the incubation medium and extracts of the pituitary tissue were assayed for prolactin content against NIAMDD rat RP1 standard. Results are expressed as prolactin released into the medium as a percentage of prolactin content of the pituitary, as a good statistical correlation has been found between these two prolactin values¹².

Table 1 shows that morphine (10^{-6} M), Met-enkephalin (10^{-6} M), β -endorphin (5×10^{-7} M) or naloxone (10^{-6} M) alone did not affect basal prolactin secretion *in vitro*. As expected, dopamine (10^{-7} M) strongly inhibited the secretion of prolactin from hemipituitaries. The three opiates tested significantly

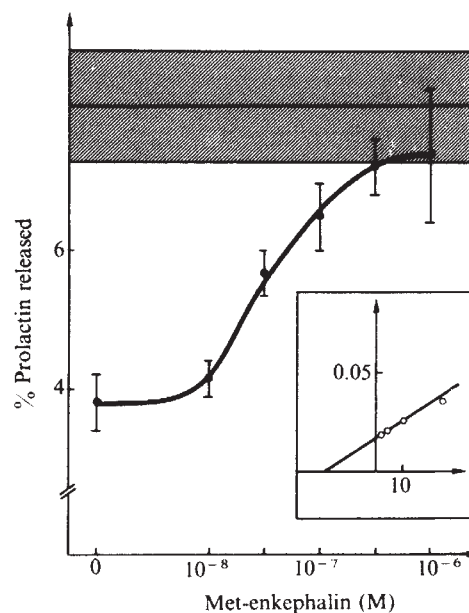


Fig. 1 Dose-response curve for the effect of Met-enkephalin on dopamine inhibition of prolactin release. The shaded area corresponds to the mean prolactin release of control pituitary $\pm$ s.e.m. Data are expressed as the percentage of the hormone content of the tissue released into the medium. Inset, Lineweaver-Burk plot of the data; ordinate: reciprocal of prolactin released; abscissa: reciprocal of Met-enkephalin concentration (μM^{-1}). Each value is the mean $\pm$ s.e.m. of 16 determinations. Average prolactin release of corresponding controls was 414 ± 36 ng ml⁻¹. In the presence of dopamine (10^{-7} M) increasing concentrations of Met-enkephalin progressively restored prolactin release to control levels.

blocked the dopamine inhibition. Their action could be antagonised by naloxone, which had no direct effect on the inhibition of prolactin secretion by the amine.

The effect of Met-enkephalin and β -endorphin on dopamine inhibition of prolactin release is dose dependent (Figs 1, 2). In the case of Met-enkephalin we were able to calculate kinetic constants for this interaction according to the method of Parker and Wand¹³. The apparent affinity was $4.1 \pm 0.8 \times 10^{-8}$ M. Complete reversal of dopamine inhibition occurred when 10^{-6} M Met-enkephalin was added to the incubation medium. The Hill constant calculated from the Met-enkephalin inhibition curve ($n = 1.2 \pm 0.3$) was indicative of simple Michaelis-Menten kinetics. A similar result was obtained using the Lineweaver-Burk¹⁴ plot of the data (Fig. 1), giving an apparent affinity of 4.5×10^{-8} M. Naloxone antagonised the effect of Met-enkephalin on dopamine inhibition in a dose-related manner (Table 2). The dopamine effect antagonised by 10^{-7} M Met-enkephalin was completely restored in the presence of 10^{-7} M naloxone. Another opiate antagonist, Mr 2266 (ref. 15), had a similar effect when added to the medium at a concentration of 10^{-7} M. In contrast, the optical isomer of this antagonist, Mr 2267 (ref. 15), was ineffective in the same conditions (Table 2).

We have thus confirmed previous data showing that incubation of pituitaries with opiates does not affect spontaneous prolactin release. This has led several authors to postulate that the morphinomimetic peptides may interact with prolactin regulating mechanisms at a higher level than the pituitary, possibly by modulating the turnover rate of tubero-infundibular dopaminergic neurones¹⁶. Although the present data do not exclude this hypothesis, they demonstrate that the prolactin response to opiates can be accounted for by a direct interaction of these substances with the effect of dopamine at the level of the pituitary prolactin cell.

Opiates do not affect dopamine receptors, as shown by ³H-dihydroergocryptine binding to pituitary membrane preparations¹⁷; naloxone, morphine, Met-enkephalin and β -endorphin

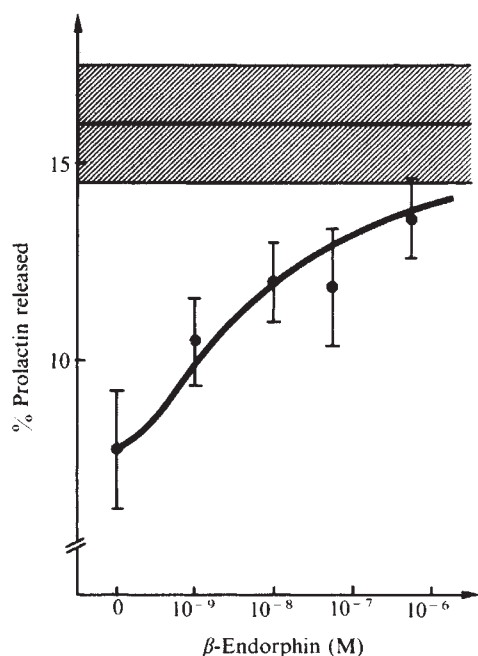


Fig. 2 Dose-response curve for the effect of β -endorphin on dopamine inhibition of prolactin release. Details as in Fig. 1 legend. Each value is the mean $\pm$ s.e.m. of eight determinations. Average prolactin release of corresponding controls was 709 ± 59 ng ml $^{-1}$.

do not affect 3 H-spiroperidol binding to striatal membranes¹⁸. Furthermore, in our experimental conditions, naloxone had no effect by itself on the dopamine-induced inhibition of prolactin release. We conclude from these results that two independent receptors mediate the action of dopamine and opiates on the pituitary.

Interactions between opiates and dopamine have already been described in other structures of the central nervous system^{19,20}. In the case of the pituitary, reversal of the opiate effect by Mr 2266 or naloxone suggests that this interaction with dopamine is mediated by specific opiate receptors. In addition, as already reported for studies using different conditions^{15,21}, ineffectiveness of the optical isomer Mr 2267 to antagonise the action of Met-enkephalin demonstrates that the interaction of Mr 2266 with opiate receptors is stereospecific. Suppression of the opiate effect with low doses of antagonists also suggests that

Table 1 Interaction of morphine, Met-enkephalin, β -endorphin and/or naloxone with basal and dopamine-inhibited release of prolactin from incubated hemi-pituitaries

	Naloxone	
	0	10^{-6} M
Control	15.1 ± 0.8	14.5 ± 0.7
Dopamine 10^{-7} M	$8.1 \pm 1.5^*$	$9.0 \pm 1.0^*$
Morphine 10^{-6} M	14.6 ± 0.9	
Dopamine + M	$14.1 \pm 1.4^\dagger$	$10.6 \pm 1.0§$
Met-enkephalin 10^{-6} M	14.4 ± 1.0	
Dopamine + Met-enkephalin	$13.9 \pm 1.6^\dagger$	$9.4 \pm 1.1§$
β -endorphin 5×10^{-7} M	14.8 ± 1.1	
Dopamine + β -endorphin	$14.0 \pm 1.0^\ddagger$	$9.2 \pm 1.4§$

Prolactin release is expressed as per cent of the hormone content of the tissue. Significance of the differences was assessed by variance analysis: *, highly significant inhibition as compared with controls; †, significant, or ‡, highly significant interaction of opiate agonists with dopamine inhibition; §, significant effect of naloxone as compared with corresponding dopamine + opiate agonist-treated hemi-pituitaries. Each value is the mean $\pm$ s.e.m. of eight determinations. Average prolactin release of corresponding controls was 722 ± 50 ng ml $^{-1}$.

Table 2 Dose dependence and stereospecificity of the effect of opiate antagonists on Met-enkephalin interaction with dopamine-inhibited prolactin release

Dopamine	Opiate antagonists	Met-enkephalin	
		0	10^{-7} M
0	0	11.2 ± 1.1	—
10^{-7} M	0	$6.2 \pm 0.5^*$	$9.3 \pm 0.2^\ddagger$
0	Naloxone 10^{-8} M	10.2 ± 0.9	—
10^{-7} M	Naloxone 10^{-8} M	—	9.5 ± 1.2
0	Naloxone 10^{-7} M	10.8 ± 0.7	—
10^{-7} M	Naloxone 10^{-7} M	—	$6.7 \pm 0.9§$
0	Naloxone 10^{-6} M	10.8 ± 0.8	—
10^{-7} M	Naloxone 10^{-6} M	$6.7 \pm 0.5^*$	$7.3 \pm 0.5§$
0	0	14.0 ± 0.7	—
10^{-7} M	0	$8.1 \pm 0.8^*$	$11.0 \pm 0.6^\ddagger$
10^{-7} M	Mr 2266 10^{-7} M	—	$8.2 \pm 0.9§$
10^{-7} M	Mr 2267 10^{-7} M	—	11.6 ± 0.3

Increasing doses of naloxone restored dopamine (10^{-7} M) inhibition in the presence of Met-enkephalin (10^{-7} M). Another opiate antagonist, Mr 2266, was only effective at a concentration of 10^{-7} M, whereas its optical isomer Mr 2267 was inactive. Statistical data are indicated with the same symbols as in Table 1. Each value is the mean $\pm$ s.e.m. of eight determinations. Average prolactin release of corresponding controls was 393 ± 39 ng ml $^{-1}$.

a specific opiate receptor is involved; interaction with other receptors only occurs with much higher concentrations of antagonists. For example, naloxone displaces 3 H-GABA binding in the cerebellum with an IC_{50} of 3×10^{-4} M (ref. 22). *In vivo* experiments using several enkephalin analogues also suggested that the effect of prolactin release is mediated by a specific opiate receptor^{7,8}.

Binding sites for morphinomimetic peptides have been identified in both the central and the peripheral nervous systems^{23,24}. The affinity of various opiate antagonists depends on the structure on which the receptor is located. Opiate receptors have been identified in the pituitary²⁵, where the receptor density is much lower in the anterior lobe than in the neurohypophysis. The small proportion of prolactin cells in normal male pituitaries where, according to the present data, these receptors might be located, may account for the small number of receptors. The affinity of opiates for this receptor was lower than that calculated for central receptors which is in the range of 10^{-9} M. Our results agree with these data, for the apparent affinity for Met-enkephalin in our system is approximately 4×10^{-8} M.

Endogenous opiates, released either by the median eminence into the hypothalamo-hypophyseal portal system or by the pituitary into the general circulation, may be involved in the physiological regulation of prolactin secretion, as stress-induced prolactin release is blocked by naloxone^{26,27} and naltrexone⁹. The opiate-dopamine interaction at the hypophyseal level which we have demonstrated may account for this physiological regulation.

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1. Bruni, G. F., Van Vugt, D., Marshall, S. & Meites, J. *Life Sci.* **21**, 461–466 (1977).
2. Rivier, C., Vale, W., Ling, N., Brown, M. & Guillemin, R. *Endocrinology* **100**, 238–241 (1977).
3. Shaar, C. J., Frederickson, C. A., Dininger, N. B. & Jackson, L. *Life Sci.* **21**, 853–860 (1977).
4. Rivier, C., Brown, M. & Vale, W. *Endocrinology* **100**, 751–754 (1977).
5. Dupont, A., Cusan, L., Labrie, F., Coy, D. H. & Li, C. H. *Biochem. biophys. Res. Commun.* **75**, 76–82 (1977).
6. Chihara, K., Arimura, A., Coy, D. H. & Schally, A. V. *Endocrinology* **102**, 281–290 (1978).
7. Lien, E. L., Clark, D. E. & McGregor, W. H. *FEBS Lett.* **88**, 201–208 (1978).
8. Meltzer, W. Y., Miller, R. J., Fessler, R. G., Simonovic, M. & Fang, V. S. *Life Sci.* **22**, 1931–1938 (1978).
9. Grandison, L. & Guidotti, I. *Nature* **270**, 357–358 (1977).
10. Enjalbert, A. *et al. Eur. J. Pharmac.* **53**, 211–212 (1979).
11. Pathy, A., Graf, L., Kenessey, A., Szekely, G. I. & Bajusz, S. *Biochem. biophys. Res. Commun.* **79**, 254–259 (1977).
12. Enjalbert, A., Moos, F., Carbonell, L., Priam, M. & Kordon, C. *Neuroendocrinology* **24**, 147–161 (1977).
13. Parker, R. B. & Wand, D. R. *Eur. J. Pharmac. exp. Ther.* **177**, 1–12.
14. Lineweaver, H. & Burk, D. J. *Am. chem. Soc.* **56**, 658–666 (1934).
15. Höllt, V. & Hertz, A. in *Opiates and Endogenous Opioid Peptides* (ed. Kosterlitz, H. W.) 345–352 (Elsevier, Amsterdam, 1976).
16. Ferland, L., Fuxe, K., Enroth, P., Gustafsson, J. A. & Skett, P. *Eur. J. Pharmac.* **43**, 89–90 (1977).
17. Caron, M. G. *et al. J. biol. Chem.* **253**, 2244–2253 (1978).
18. Czlonkowska, A., Höllt, V. & Herz, A. *Life Sci.* **22**, 953–962 (1978).
19. Pollard, H., Llorens-Cortes, C. & Schwartz, J. C. *Nature* **268**, 745–747 (1977).
20. Rotsztein, W. H., Drouva, S. V., Pattou, E. & Kordon, C. *Nature* **274**, 281–282 (1978).
21. Jacob J. J. C. & Ramabadran, K. *Br. J. Pharmac.* **64**, 91–98 (1978).
22. Dingledine, R., Iversen, L. L. & Breuker, E. *Eur. J. Pharmac.* **47**, 19–27 (1978).
23. Lord, J. A. H., Waterfield, A. A., Hughes, J. & Kosterlitz, H. W. in *Opiates and Endogenous Opioid Peptides* (ed. Kosterlitz, H. W.) 275–280 (Elsevier, Amsterdam, 1976).
24. Della Bella, D., Casacci, F. & Sassi, A. *Adv. biochem. Psychopharmac.* **18**, 271–278 (1978).
25. Simantov, R. & Snyder, S. H. *Brain Res.* **124**, 178–184 (1977).
26. Van Vugt, D. A., Bruni, G. F. & Meites, J. *Life Sci.* **22**, 85–90 (1977).
27. Ferland, L., Kledzik, G. S., Cusan, L. & Labrie, F. *Molec. cell. Endocr.* **12**, 267–272 (1978).

Electrical properties of giant mitochondria studied with a double impalement technique

THE electrical properties of the membrane of giant mitochondria (4–10 μm in diameter) can be studied directly with microelectrodes. Tupper and Tedeschi^{1–4} have studied mitochondria isolated from *Drosophila* (approximately 4 μm in diameter), and found a positive potential in the range of 5–15 mV and a specific resistance in the range of 1–4 Ωcm^2 (assuming no invaginations of the mitochondrial membrane). The potentials were not found to depend on metabolic state². The microelectrodes were judged to be in the internal mitochondrial space from the fact that the potentials were osmotically sensitive (decreasing with osmotic swelling in a predictable manner). The positive potentials correlated with the distribution of carboxylic anions, which partition with a higher concentration inside the mitochondria. Furthermore, the potentials were collapsed by the addition of acetate^{3,4}, but not chloride⁴ to the external medium. Presumably acetate enters and depolarises the potential. These observations further support the idea that the electrodes are in the inner mitochondrial space.

More recently, we have carried out studies with giant mitochondria from mice maintained on a diet containing cuprizone^{5–8}. The isolated giant mitochondria seem to be in good physiological condition, as shown by the respiratory control of single isolated mitochondria⁹, the P:O ratios of the mitochondrial suspensions^{7,10}, the direct bioassay of the ATP produced by single mitochondria or the ability to accumulate calcium phosphate^{7,11}. The potentials were found to be in the range of 10–20 mV (positive inside) over a wide range of ionic composition of the medium. The specific resistances were found to be in the range of 1–5 Ωcm^2 (neglecting the membrane invaginations). The microelectrodes were in the inner mitochondrial space as shown by the following criteria⁶. (1) In the presence of valinomycin, the potentials reversed in polarity and became sensitive to the K^+ concentration of the medium. Over a range of external K^+ concentrations, the measured potentials followed the Nernst equation approximately. (2) Almost identical results were obtained with mitochondria from which the external

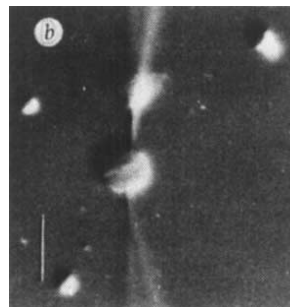
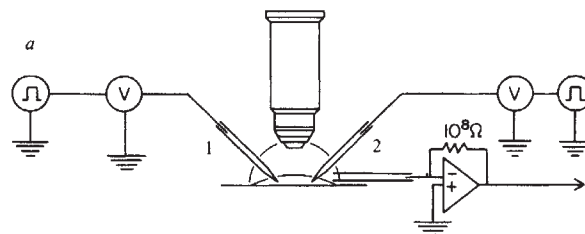


Fig. 1 *a*, Diagrammatic representation of the experimental design. 1 and 2 correspond to the electrodes used. The resistor shown is 10^7 or $10^8\ \Omega$. The capacitor corresponds to 0–20 pF. *b*, A doubly impaled mitochondrion seen with Nomarski optics, water immersion objective (see ref. 6). The bar indicates 10 μm .

membrane was removed. (3) In a variety of conditions, advancing the electrode through the mitochondrion revealed a single potential, as long as the microelectrode had not emerged to the other side.

The measured positive potentials cannot be ascribed to artefacts involving the electrode tip, as the results were the same, regardless of whether the microelectrodes were loaded with KCl, NaCl (ref. 8) or potassium acetate (unpublished results).

Individual, intact or impaled mitochondria were found to be metabolically viable by two kinds of assays⁷. In one assay, the individual mitochondria were shown to exhibit granules in conditions in which mitochondria were known to accumulate massive amounts of calcium phosphate. The granulations were shown to correlate with increases in dry mass. They were interpreted to correspond to sites of accumulation of calcium phosphate. In another assay, individual mitochondria were shown to be capable of inducing the contraction of glycerinated myofibrils in close proximity to the mitochondrion (in some cases in the absence of other mitochondria¹¹). Both the apparent

Table 1 Resistances after using the techniques represented in the figures

Experiments	Experimental design	Resistances (M Ω)
Series 1		
1	Single impalement (<i>b</i>)	3.7 ± 1.1 ($n = 12$)
2	Double impalement; measurement with the electrode delivering the current (<i>c</i>)	1.9 ± 0.8 ($n = 12$)
3	Double impalement; measurement with the electrode not delivering the current (<i>c</i>)	2.0 ± 1.1 ($n = 12$)
Series 2		
1	Single impalement (<i>b</i>)	3.8 ± 2.6 ($n = 6$)
2	Double impalement; measurement with electrode* delivering the current (<i>c</i>)	2.9 ± 2.5 ($n = 6$) 3.2 ± 2.0 ($n = 6$)
3	Double impalement; measurement with electrode* not delivering the current (<i>c</i>)	4.6 ± 2.4 ($n = 6$) 4.5 ± 2.2 ($n = 6$)

Values listed represent means $\pm$ s.d. The letters correspond to the diagram of Fig. 2. For series 1 the mitochondria were in 0.30 molal sucrose, 40 mM KCl, 1 mM MgCl_2 , 3 mM sodium succinate, 2 mM potassium phosphate and 5 mM HEPES, pH 7.3. Excluding one value (-13 mV) for series 1, the membrane potentials were 1.2 ± 1.8 mV. For series 2, the mitochondria were in 0.30 molal sucrose, 10 mM KCl and 5 mM HEPES, pH 7.3. Only one membrane potential (+5 mV) was recorded in series 2.

* The induced potential was recorded from both electrodes.

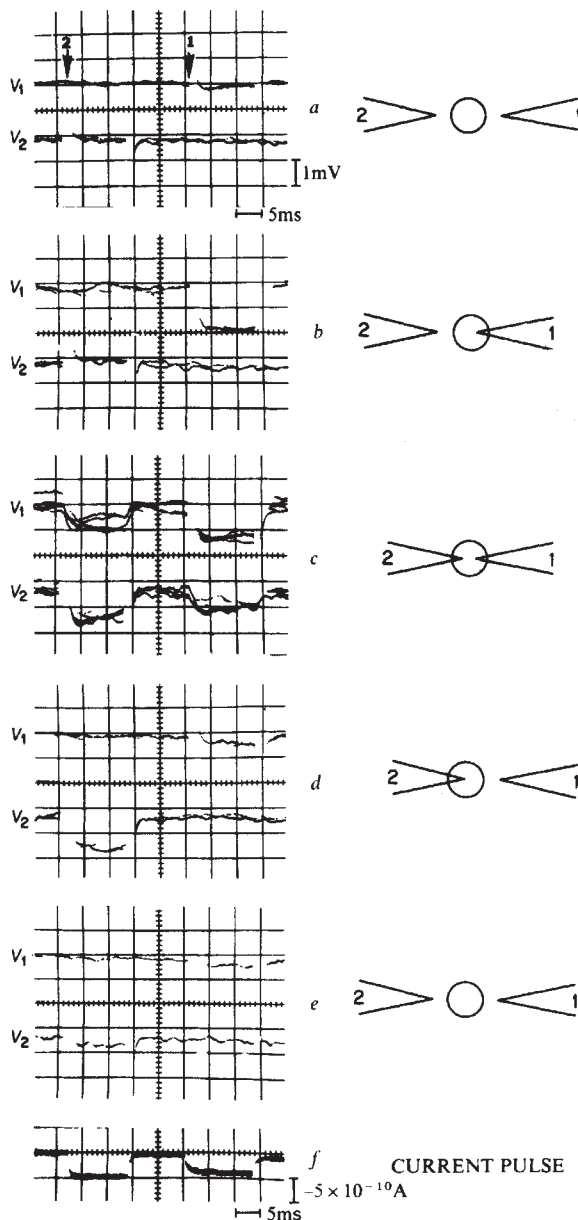


Fig. 2 The position of the electrodes and the electrical recording from a mitochondrion. The oscilloscope tracings are indicated on the left. The arrows indicate the start of the current pulses. The contrast has been reversed for ease in viewing. The corresponding location of the microelectrodes are indicated by the diagrams on the right. The microelectrodes are conventional micropipettes filled with 2 M KCl. The ground electrode consisted of a glass pipette containing 2 M KCl in 2% agar with a Ag-AgCl wire inside and connected to the virtual ground amplifier. Two electrometers (W-P Instruments, model M4A) with a minimum input resistance of $10^{10} \Omega$ were used to pass current and simultaneously record potentials. The bath was held at virtual ground with the reference electrode by means of a current to voltage amplifier (Burr-Brown, operational amplifier, model 3308/12C) using a feedback resistor of either 10^7 or $10^8 \Omega$ (wire wound, Victoreen). The output of this amplifier was monitored by a Tektronix oscilloscope model 5110, using a 5A20N differential amplifier and a 5B10N time base (Tektronix). The output of electrometers was recorded with a Tektronix storage oscilloscope type R 564 B using a type 3A3 dual-trace differential amplifier and a type 2B67 time base. Current was injected using two type 161 Tektronix pulse generators controlled by a type 162 waveform generator. In this experiment negative current pulses of -4×10^{-10} A were delivered from electrode 1 and -3.5×10^{-10} A from electrode 2. The mitochondrion (8 μ m in diameter) was in 0.3 molar sucrose, 10 mM KCl and 5 mM HEPES, pH 7.3 ($\approx 25^\circ \text{C}$).

accumulation of calcium phosphate and the contraction of the myofibrils were sensitive to the appropriate inhibitors or uncouplers of oxidative phosphorylation.

Despite the evidence summarised above, some investigators seem to harbour considerable reservation about the location of the microelectrodes. We have carried out a new series of experiments that bear on this question. Electrical tests of the location of microelectrodes in doubly impaled cells^{12,13} or nuclei¹⁴ have been in use for some time in electrophysiology. Characteristic voltage changes can be obtained with the delivery of current, depending on the location of the microelectrodes. In these experiments one microelectrode is used to deliver current and another to record potential. The experimental design allows one to distinguish the different possible locations of the microelectrodes from a record of the potential changes. In the case of cells, for example, one can distinguish whether both electrodes are outside the cell, both electrodes are pressed against the cells, (in the cases studied, the procedure actually produces deep invaginations where the electrodes are pressed against the cell surface (see Fig. 1 or ref. 12)), or both electrodes are inside the cell (see, for example, Fig. 4 of ref. 12). Only the latter experimental situation demonstrates a significant voltage response.

The microelectrodes and the pertinent circuits of our experiments are represented in Fig. 1a. The design allows recording and/or current delivery from either electrode. A doubly impaled mitochondrion is shown in Fig. 1b. The tracings correspond to oscilloscope records. Generally we used microelectrodes approximately 70 M Ω in tip resistance. In previous studies we have found that the results were independent of the tip resistance in the range of 10 to approximately 80 M Ω (refs 1 and 5). The records indicated in Fig. 2 were obtained after an initial balancing of the tip resistance. The impalements and withdrawals were sequential from a to e. f represents the current pulses, the first delivered from electrode 2 (-4×10^{-10} A), the second from electrode 1 (-3.5×10^{-10} A). In the oscilloscope record corresponding to the double impalement (c), five separate pulses have been superimposed. When both electrodes are in the external medium (a or e) there is no significant response from either electrode. We have found an absence of an induced potential at these current levels even when the electrodes are held at a distance well below 1 μ m. With a single impalement (b and d), a response is obtained only from the impaling electrode. When both electrodes are in the mitochondrion (c) either electrode records the potential regardless of which delivers the current. Table 1 summarises two series of comparable experiments. The potentials across the mitochondrial membrane are in the legend to Table 1.

These experiments demonstrate that the two microelectrodes impaling a mitochondrion are in the same membrane-bounded space. Previous studies⁶ have shown the same resistances in the presence and in the absence of the mitochondrial outer membrane, suggesting that in the present experiments the two electrodes are in the inner mitochondrial space.

These experiments and those previously presented¹⁻⁸ constitute evidence that the electrodes are in the mitochondrial matrix space and indicate the absence of a significant role of the membrane potential in the coupling of oxidation to phosphorylation. The details of a study on the membrane resistance of mitochondria using the double impalement technique will be presented elsewhere.

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1. Tupper, J. T. & Tedeschi, H. *Proc. natn. Acad. Sci. U.S.A.* **63**, 370–377 (1969).
2. Tupper, J. T. & Tedeschi, H. *Proc. natn. Acad. Sci. U.S.A.* **63**, 713–717 (1969).
3. Tupper, J. T. & Tedeschi, H. *Science* **166**, 1539–1540 (1969).
4. Tedeschi, H. in *Energy Transduction in Respiration and Photosynthesis* (eds Quagliariello, E., Papa, S. & Rossi, C. S.) 767–779 (Adriatica Editrice, Bari, 1971).
5. Maloff, B. L., Scordilis, S. P. & Tedeschi, H. *Science* **195**, 898–900 (1975).
6. Maloff, B. L., Scordilis, S. P. & Tedeschi, H. *J. Cell Biol.* **78**, 199–213 (1978).
7. Maloff, B. L., Scordilis, S. P. & Tedeschi, H. *J. Cell Biol.* **78**, 214–226 (1978).
8. Maloff, B. L., Scordilis, S. P. & Tedeschi, H. *Science* **199**, 568–569 (1978).
9. Suchy, J. & Cooper, C. *Expl. cell. Res.* **88**, 198–202 (1974).
10. Wakabayashi, T., Asano, M. & Kuroso, C. *Acta path. Jap.* **25**, 39–49 (1975).
11. Bowman, C., Maloff, B. L. & Tedeschi, H. in *Frontiers of Biological Energetics 1* (eds Dutton, P. L., Leigh, J. & Scarpa, A.) 413–421 (Academic, New York, 1978).
12. Tyler, A., Monroy, A., Kao, C. Y. & Grundfest, H. *Biol. Bull.* **111**, 153–177 (1956).
13. Loewenstein, W. R. & Kanno, Y. *J. Cell Biol.* **22**, 565–586 (1964).
14. Loewenstein, W. R. & Kanno, Y. *J. gen. Physiol.* **46**, 1123–1140 (1963).

Use of Panamanian sea urchins to test the molecular clock

THE 'molecular clock' hypothesis of protein evolution holds that each protein changes at a constant rate, so that the degree of molecular divergence between two species is linearly related to the time for which their lineages have remained separate¹. This assertion, however, has been challenged repeatedly by authors who discovered taxa and peptides in which the proposed uniformity of molecular evolution did not hold^{2,3}, who noted that biochemically and palaeontologically determined dates of separation between lineages conflicted^{4,5}, introduced tests that pointed to significant variation in the rates of evolution of the same proteins^{6,7}, or dismissed the hypothesis as a confusion of averages with constants⁸. Others have postulated that, although the same proteins evolve at different rates in different lineages, the average amount of molecular change over many proteins is sufficiently uniform to provide approximate dates for the splitting of two lines of descent^{9,10}. Here I present evidence from sea urchins separated by the Isthmus of Panama which indicates that even this compromise position is not tenable.

It is important to resolve this issue for two reasons: first, the assumption of a linear relationship between time since separation and degree of molecular divergence has been used extensively to assign dates to the splitting of two lineages on the basis of biochemical evidence^{11–15} and second, the proposed constancy of rate of amino acid substitution has frequently been cited as the strongest evidence for the neutral mutation theory, which maintains that the major cause of molecular evolution is random fixation of selectively neutral mutations^{16–19}. In spite of one model that tries to account for the phenomenon in terms of natural selection²⁰, the most parsimonious theoretical basis for such a uniformity of rates remains that proposed by Kimura¹⁷. If most amino acid substitutions were neutral, their rate of fixation would equal the mutation rate, and it would be constant over long periods of time, provided that mutations are random events.

Because of the uncertainties involved in assigning fossils to either branch of a phylogenetic tree if they lie close to the node, rate determinations that use dates based on palaeontological evidence have been challenged by both proponents^{1,14,15,21} and opponents²² of the molecular clock. The best way to test the clock hypothesis is to assess the relative amounts of divergence within two or more groups, all of which split into new lines of descent at the same time¹. Such a situation holds for the species pairs of Panama¹¹, where the emergence of the Isthmus in the Pliocene^{23–25} fragmented the ranges of many marine species simultaneously. Populations thus separated have become known as geminate species.

There are seven genera of shallow water regular echinoids in the Caribbean, each of which is also represented in the eastern Pacific by populations that have been assigned a different specific name. On morphological grounds the Atlantic and eastern Pacific members of each genus are presumed to comprise geminate pairs²⁶. I compared three pairs: *Diadema antillarum* (Caribbean (C)) and *Diadema mexicanum* (Pacific

(P)); *Eucidaris tribuloides* (C) and *Eucidaris thouarsi* (P); *Echinometra lucunter* (C) and *Echinometra vanbrunti* (P). *Echinometra viridis*, a species sympatric with *E. lucunter*, was also included, because of the uncertainty as to which of the two is the true geminate of their Pacific counterpart. Enzymatic proteins were compared by electrophoresis; 18 presumptive loci (15 for *Eucidaris*) were sampled for two populations per species. Trans-isthmian divergence is measured relative to differentiation between conspecific population on the same coast. Such a calibration corrects for the possibility that electrophoretically undetected heterogeneity²⁷ may be unequally distributed among the genera or that the enzymes assayed are a nonrandom sample of the existing rates of molecular evolution. Differences are quantified by Nei's²⁸ standard genetic distance.

The clock hypothesis predicts that the extent of molecular differentiation in all pairs should be equivalent. Clearly, this is not the case (Table 1). Pacific populations of *Diadema* have diverged from their Atlantic counterparts no more than they have from populations on the same coast. *Eucidaris* and *Echinometra*, on the other hand, exhibit trans-isthmian distances 16 and 37 times greater than intraspecific ones. Even if we take the conservative stand that intraspecific distances are roughly the same for all genera, we find that the values of Nei's index for inter-oceanic comparisons of *Echinometra* are more than 20 times larger than those for *Diadema*. This conclusion holds whether *E. vanbrunti* is compared with either *E. lucunter* or *E. viridis*.

It is unlikely that the determined dissimilarities in divergence between geminates in the three genera are artefacts of the limited resolving power of electrophoresis. If 'hidden variation' in *Diadema* were 20 times lower than in *Echinometra*, we would expect the former also to exhibit lower values of intraspecific differentiation and heterozygosity. No such trend is apparent (Tables 1, 2).

The 20-fold difference between *Echinometra* and *Diadema* is also too great to be the result of different times of isolation during the gradual closing of the portals connecting the two oceans. All three genera produce planktonic larvae²⁹, and cessation of gene flow between their members must have been almost simultaneous. Furthermore, although the duration of the actual geological event is unknown, there is a range of estimates for the date of final completion of the land bridge between North and South America; the highest currently accepted estimate is 3.5–5.7 Myr ago^{23,30}, and the lowest about 2 Myr ago^{24,25}. If we place the separation of *E. vanbrunti* from *E. lucunter* (or *E. viridis*) at this date and accept that divergence is linearly related to time, the analogous event in *Diadema* would have to be dated at 100,000 to 285,000 yr ago, when the land bridge between North and South America was continuous²⁵. Similarly, the postulate that *Diadema* split at the time the Isthmus rose would require the speciation event in *Echinometra* to have occurred between the Eocene and the Cretaceous. Such an early origin of the extant species, whether due to the emergence of the Isthmus or some other geological occurrence, is unlikely as, despite a good echinoid fossil record, the genus is unknown before the Pliocene in the eastern Pacific³¹ and the Oligocene in the Caribbean³².

The disparity of the genetic distance values is also too great to be due to the stochastic variation allowed by proponents of the clock^{1,19}. Nor can it be the result of generation time³³, as *Diadema* reaches sexual maturity sooner than *Eucidaris* or *Echinometra*³⁴.

The possibility that the extant species of *Echinometra* and *Eucidaris* may have speciated after the rise of the Isthmus from true geminates which subsequently became extinct would not redeem the clock hypothesis as molecular clocks are based on the assumption that divergence is dependent on the time two lineages have remained separate and not on the number of speciation events in each.

Different amounts of divergence between the species of *Diadema*, *Eucidaris* and *Echinometra* must, therefore, reflect

Table 1 Nei's standard genetic distance (D) between populations in each genus $\pm$ s.e.

Species	Locality	Atlantic				Pacific
		<i>E. lucunter</i> Maria Chiquita	B. del Toro	<i>E. viridis</i> San Blas Is	B. del Toro	<i>E. vanbrunti</i> P. Paitilla
<i>E. lucunter</i>	B. del Toro	0.009 ± 0.004 (0.012 ± 0.006)				
<i>E. viridis</i>	San Blas Is	0.117 ± 0.075 (0.180 ± 0.121)	0.109 ± 0.073 (0.169 ± 0.119)			
	B. del Toro	0.117 ± 0.073 (0.177 ± 0.120)	0.111 ± 0.072 (0.172 ± 0.119)	0.007 ± 0.003 (0.008 ± 0.005)		
<i>E. vanbrunti</i>	P. Paitilla	0.557 ± 0.209 (0.655 ± 0.288)	0.531 ± 0.203 (0.649 ± 0.290)	0.620 ± 0.218 (0.771 ± 0.303)	0.612 ± 0.214 (0.790 ± 0.304)	
	Isla Uva	0.561 ± 0.213 (0.658 ± 0.295)	0.547 ± 0.209 (0.672 ± 0.299)	0.666 ± 0.233 (0.847 ± 0.333)	0.653 ± 0.225 (0.854 ± 0.328)	0.021 ± 0.011 (0.032 ± 0.018)
<i>E. lucunter</i> – <i>E. vanbrunti</i>						
Mean intraspecific genetic distance:		0.015 (0.022)				
Mean transisthmian genetic distance:		0.549 (0.659)				
<i>E. viridis</i> – <i>E. vanbrunti</i>						
Mean intraspecific genetic distance:		0.014 (0.010)				
Mean transisthmian genetic distance:		0.638 (0.816)				
		<i>E. tribuloides</i>				<i>E. thourarsi</i>
		San Blas Is	B. del Toro			P. Paitilla
<i>E. tribuloides</i>	B. del Toro	0.016 ± 0.014 (0.021 ± 0.018)				
<i>E. thourarsi</i>	P. Paitilla	0.292 ± 0.143 (0.400 ± 0.193)	0.307 ± 0.150 (0.419 ± 0.202)			
	Isla Uva	0.357 ± 0.166 (0.480 ± 0.221)	0.360 ± 0.166 (0.482 ± 0.221)			0.024 ± 0.012 (0.028 ± 0.015)
Mean intraspecific genetic distance:		0.020 (0.025)				
Mean transisthmian genetic distance:		0.329 (0.445)				
		<i>D. antillarum</i>				<i>D. mexicanum</i>
		Ft Randolph	B. del Toro			Isla Uraba
<i>D. antillarum</i>	B. del Toro	0.036 ± 0.016 (0.038 ± 0.019)				
<i>D. mexicanum</i>	Is Uraba	0.040 ± 0.026 (0.052 ± 0.040)	0.016 ± 0.007 (0.023 ± 0.011)			
	Isla Uva	0.039 ± 0.017 (0.046 ± 0.023)	0.008 ± 0.004 (0.012 ± 0.007)			0.015 ± 0.007 (0.024 ± 0.010)
Mean intraspecific genetic distance:		0.026 (0.031)				
Mean transisthmian genetic distance:		0.026 (0.033)				

Values in parentheses are those obtained from an analysis restricted to the 12 loci common to all three genera. Electrophoresis was carried out on 11% starch for all enzymes except amylase; 7% acrylamide gels were used for the latter. Standard histochemical techniques were used for staining. Collections were made at: Fort Randolph, Canal Zone (*D. antillarum*); Bocas del Toro, Republic of Panama (*D. antillarum*, *E. tribuloides*, *E. viridis*, *E. lucunter*); Maria Chiquita, Republic of Panama (*E. lucunter*); San Blas Islands, Republic of Panama (*E. viridis*, *E. tribuloides*); Isla Uraba, Bay of Panama (*D. mexicanum*); Punta Paitilla, Bay of Panama (*E. thourarsi*, *E. vanbrunti*); Isla Uva, Gulf of Chiriqui (*D. mexicanum*, *E. thourarsi*, *E. vanbrunti*). Loci examined for each genus were: *Diadema*: phosphoglucosylase (PGM): PGM-1, PGM-2; phosphoglucose isomerase (PGI); hexokinase (HK); peptidases (Pep): Pep-1, Pep-2, mannose-6-phosphate isomerase (M6PI); NAD-dependent malate dehydrogenase (MDH): MDH-1, MDH-2, triose phosphate isomerase (TPI); leucine amino peptidase (LAP); amylase (Am): Am-1, Am-2; tetrazolium oxidase (TO); glucose-6-phosphate dehydrogenase (G6PDH); xanthine dehydrogenase (XDH); esterases (Est): Est-1, Est-2, Est-3. *Echinometra*: PGM-1, PGM-2, PGI, HK, Pep-1, Pep-2, M6PI, MDH-1, MDH-2, TPI, Am-1, Am-2, Am-3, TO, G6PDH, XDH, LAP-1, LAP-2. *Eucidaris*: PGM-1, PGI, HK, Pep-1, M6PI, MDH-1, TPI, LAP, AM-1, AM-2, TO, XDH, Est-1, Est-2, Est-3. Sample sizes per locus (number of alleles) ranged from 36 to 134 (most above 90), with one, PGM-1, *E. thourarsi*, Isla Uva, represented by only six. See ref 39 for details of the methods.

Table 2 Average heterozygosity (H) (Nei's²⁸ gene diversity) of populations studied $\pm$ s.e.

Species	Locality	<i>H</i>	Species	Locality	<i>H</i>
<i>E. lucunter</i>	M. Chiquita	0.254 $\pm$ 0.058 (0.278 $\pm$ 0.080)	<i>E. vanbrunti</i>	Paitilla	0.216 $\pm$ 0.056 (0.278 $\pm$ 0.076)
	B. del Toro	0.259 $\pm$ 0.058 (0.291 $\pm$ 0.081)		Isla Uva	0.182 $\pm$ 0.052 (0.229 $\pm$ 0.073)
<i>E. viridis</i>	San Blas	0.202 $\pm$ 0.065 (0.231 $\pm$ 0.093)			
	B. del Toro	0.183 $\pm$ 0.061 (0.232 $\pm$ 0.087)			
<i>E. tribuloides</i>	San Blas	0.192 $\pm$ 0.045 (0.214 $\pm$ 0.050)	<i>E. thourarsi</i>	Paitilla	0.243 $\pm$ 0.051 (0.288 $\pm$ 0.056)
	B. del Toro	0.202 $\pm$ 0.048 (0.221 $\pm$ 0.053)		Isla Uva	0.111 $\pm$ 0.036 (0.115 $\pm$ 0.040)
<i>D. antillarum</i>	Ft Randolph	0.216 $\pm$ 0.056 (0.276 $\pm$ 0.072)	<i>D. mexicanum</i>	Uraba	0.201 $\pm$ 0.056 (0.249 $\pm$ 0.072)
	B. del Toro	0.162 $\pm$ 0.051 (0.181 $\pm$ 0.068)		Isla Uva	0.184 $\pm$ 0.049 (0.217 $\pm$ 0.063)

Values in parentheses are those obtained from an analysis restricted to the 12 loci common to all three genera.

dissimilar rates of protein differentiation, and argue against the molecular clock hypothesis. West (in preparation) has reached the same conclusion on the basis of allozymic differentiation in four geminate species pairs of Panamanian crabs.

The results could still be compatible with the neutral mutation theory if they reflected differences between the genera in mutation rates or in histories of population size. Both these postulates, however, would conflict with the predictions of the same theory regarding the heterozygosity values of the populations involved.

Many models³⁵⁻³⁷ predict that variability of neutral alleles is a function of the mutation rate. If *Diadema* has a mutation rate 20 times lower than *Echinometra*, it should also display less gene diversity. This is not the case (Table 2). Average heterozygosity values are very similar in populations belonging to the two genera. A severe population restriction more recent than 10⁷ generations ago in *Echinometra* but not *Diadema* could also result in dissimilar rates of divergence compatible with the neutral mutation theory³⁸. However, if the higher rates of divergence in *Echinometra* were the result of such a bottleneck, neutral theory would expect its populations in at least one ocean to exhibit lower levels of heterozygosity than those of *Diadema*³⁸. This is not so (Table 2).

I conclude that the molecular clock hypothesis does not hold for the Panamanian echinoids, and that their unequal rates of protein divergence with similar levels of heterozygosity are in direct opposition to the predictions of at least one model based on the neutral mutation theory. The most plausible explanation for the observed discrepancies is that molecules have been evolving under the influence of natural selection.

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1. Wilson, A. C., Carlson, S. S. & White, T. J. *A. Rev. Biochem.* **46**, 573-639 (1977).
2. Goodman, M., Barnabas, J., Matsuda, G. & Moore, G. W. *Nature* **233**, 604-613 (1971).
3. Jukes, T. H. & Holmquist, R. *Science* **177**, 530-532 (1972).
4. Radinsky, L. *Science* **200**, 1182-1183 (1978).
5. Simons, E. L. *Ann. N. Y. Acad. Sci.* **167**, 319-331 (1969).
6. Langley, C. H. & Fitch, W. M. *J. molec. Evol.* **3**, 161-177 (1974).
7. Moore, G. W., Goodman, M., Callahan, C., Holmquist, R. & Moise, H. *J. molec. Biol.* **105**, 15-37 (1976).
8. Stebbins, G. L. & Lewontin, R. C. *Proc. 6th Berkeley Symp. Math. Stat. Prob.* **V**, 23-42 (1972).
9. Fitch, W. M. & Langley, C. H. *Fedn Proc.* **35**, 2092-2097 (1976).
10. Dobzhansky, T., Ayala, F. J., Stebbins, G. L. & Valentine, J. W. *Evolution* (Freeman, San Francisco, 1977).
11. Gorman, G. C., Kim, Y. J. & Rubino, R. *Copeia* **1976**, 361-364 (1976).
12. Maxson, L. R. & Wilson, A. C. *Science* **185**, 66-68 (1975).
13. Sarich, V. M. *Syst. Zool.* **18**, 416-422 (1969).
14. Sarich, V. M. & Wilson, A. C. *Science* **158**, 1200-1203 (1967).
15. Wilson, A. C. & Sarich, V. M. *Proc. natn. Acad. Sci. U.S.A.* **63**, 1088-1093 (1969).
16. Kimura, M. & Ohta, T. *J. molec. Evol.* **1**, 1-17 (1971); *Nature* **229**, 467-469 (1971); *Genetics, Princeton* **73**, Suppl. 19-35 (1973).
17. Kimura, M. *Proc. natn. Acad. Sci. U.S.A.* **63**, 1181-1188 (1969).
18. King, J. L. & Jukes, T. H. *Science* **164**, 788-798 (1969).
19. Ohta, T. & Kimura, M. *J. molec. Evol.* **1**, 18-25 (1971).
20. Van Valen, L. *J. molec. Evol.* **3**, 89-101 (1974).
21. Carlson, S. S., Wilson, A. C. & Maxson, R. D. *Science* **200**, 1183-1185 (1978).
22. Richmond, R. C. *Nature* **225**, 1025-1028 (1970).
23. Saito, T. *Geology* **4**, 305-309 (1976).
24. Webb, S. D. *Paleobiology* **2**, 220-234 (1976); *A. Rev. ecol. Syst.* **9**, 393-426 (1978).
25. Woodring, W. P. *Proc. Am. Phil. Soc.* **110**, 425-433 (1966).
26. Chesher, R. H. *Bull. biol. Soc. Wash.* **2**, 139-158 (1972).
27. Johnson, G. B. *A. Rev. ecol. Syst.* **8**, 309-328 (1977).
28. Nei, M. *Molecular Population Genetics and Evolution* (North-Holland, Amsterdam, 1975).
29. Mortensen, T. *Studies of the Development and Larval Forms of Echinoderms* (Gad, Copenhagen, 1921).
30. Emiliani, C., Gartner, S. & Lidz, B. *Paleogeogr. Paleoclim. Paleoeol.* **11**, 1-10 (1972).
31. Durham, J. W. & Allison, E. C. *Syst. Zool.* **9**, 47-91 (1960).
32. Fell, H. B. & Pawson, D. L. in *Treatise on Invertebrate Paleontology Part U*, Vol. 3 (ed. Moore, R. C.) 367-440 (Geological Society of America, Kansas, 1966).
33. Lovejoy, C. O., Bernstein, A. H. & Heipe, K. G. *Science* **176**, 803-805 (1972).
34. Hendler, G. *Proc. 3rd Int. Coral Reef Symp.* **1**, 217-223 (1977).
35. Ewens, W. J. *Genetics Princeton* **50**, 891-898 (1964).
36. Kimura, M. *Genet. Res.* **11**, 247-269 (1968); *Genetics Princeton* **61**, 893-903 (1969).
37. Ohta, T. & Kimura, M. *Genet. Res.* **22**, 201-204 (1973); *Am. Nat.* **109**, 137-145 (1975).
38. Chakraborty, R. & Nei, M. *Evolution* **31**, 347-356 (1977).
39. Lessios, H. A. thesis, Yale Univ. (1979).

Placental alkaline phosphatase in Hominidae

HUMAN placental alkaline phosphatase has several unique properties. It is stable to heating at 65 °C (ref. 1), is immunochemically distinct from the isoenzymes in other adult organs^{2,3}, and has a large number of allelic variants⁴⁻⁶, with a 'degree of heterozygosity' much larger than that of any other human enzyme studied to date⁷. If placental phosphatase is specific to fetal development, it would be logical to expect that a similar isoenzyme would be present during fetal development in other mammalian species. This is not the case, however, as several species, including rhesus monkey, African green monkey and baboon, do not have in their placentae an isoenzyme which resembles human placental phosphatase⁸. The presence of the placental isoenzyme in humans therefore seems to be a late evolutionary event. To determine the species specificity of this enzyme, we have used the criteria which distinguish the human placental isoenzyme, including inhibition by 5 mM L-phenylalanine¹ and insensitivity to inhibition by 0.1 mM bromotetramisole⁹, to compare the enzymes in species closely related to man. We report here that of the species studied, only the chimpanzee and orangutan have an isoenzyme resembling that in humans.

The enzyme assay used was as previously described¹⁰. Placental homogenates using full-term placentae were prepared using 3 ml of 0.01 M Tris-HCl, pH 7.6, per g of tissue. Antiserum used was prepared as described previously¹¹ and was obtained 10 weeks after primary immunisation. Enzyme staining was also as previously described¹¹.

Table 1 summarises the properties of the primate placental phosphatases. The enzymes from human, chimpanzee and orangutan placentae are similar in their activity, sensitivity to bromotetramisole and insensitivity to heat inactivation. The chimpanzee and human placental phosphatases are also very similar in their sensitivity to L-phenylalanine inhibition, the orangutan enzyme, less so.

The gorilla and spider monkey placental phosphatases were less active than those of the other three by two to three orders of magnitude, and were more sensitive to inhibition by bromo-

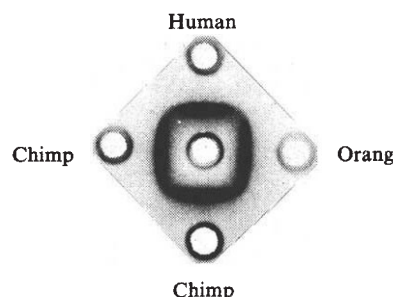


Fig. 1 Double diffusion of human, chimpanzee (chimp) and Bornean orangutan (orang) placental alkaline phosphatases against monospecific antiserum raised against human placental phosphatase. Each enzyme, 0.012 units, was placed into the appropriate wells and 10 µl of 1:8 diluted antiserum was placed in the centre well. Diffusion was allowed to proceed for 3 days at 37 °C, after which the gel was exhaustively washed with 0.01 M Tris-buffered saline, pH 7.6, and stained for enzyme.

Table 1 Properties of alkaline phosphatases from several primates

	Activity ($\mu\text{mol min}^{-1} \text{ml}^{-1}$)	Per cent inactivation by:		
		Br-tet	L-Phe	Heat
Human	5.7	32 $\pm$ 7	77 $\pm$ 5	6 $\pm$ 5
Chimpanzee	8.0	30 $\pm$ 7	70 $\pm$ 2	6 $\pm$ 1
Lowland gorilla	0.01	80 $\pm$ 2	31 $\pm$ 7	77 $\pm$ 4
Orangutan	2.7	35 $\pm$ 12	45 $\pm$ 10	15 $\pm$ 3
Spider monkey	0.04	56 $\pm$ 3	30 $\pm$ 6	62 $\pm$ 4

Br-tet, 0.1 mM bromotetramisole (Aldrich); L-Phe, 5.0 mM L-phenylalanine (Sigma); heat, heating for 10 min at 65 °C.

tetramisole, less sensitive to L-phenylalanine inhibition and markedly more readily inactivated by heat. The orangutan placental phosphatase was the least sensitive to L-phenylalanine inhibition.

Note that 20% of the lowland gorilla placental phosphatase and 38% of the spider monkey enzyme remained after heating for 10 min at 65 °C. In both cases this remaining activity was three orders of magnitude less than the amount of heat-stable enzyme found in the human, chimpanzee and orangutan placentae. Figure 1 shows the results of comparing the three related placentae by double diffusion. The absence of 'spurs' indicates that these enzymes are similar in their immunochemical structure.

The considerable difference of the human, chimpanzee and orangutan placental phosphatases from those of baboon, rhesus monkey, African green monkey and spider monkey suggests that the occurrence of the type and/or amount of alkaline phosphatase found in human placentae is a relatively late event in primate evolution. The apparent absence of a similar isoenzyme in lowland gorillas is surprising, as there is no evidence¹² for a divergence of this species from the line of man's descent after the orangutan. However, this placenta was only a single sample, and was taken from a mother with abruptio placentae and recent fetal death. The content of luteinising hormone in the placenta and its histology were normal.

The existence of a null allele for human placental phosphatase has been suggested¹³. The very low activity of the enzyme in the lowland gorilla could be due to the infant being homozygous for this null allele. Alternatively, there could be an alteration in the regulation of synthesis of the enzyme in this species, so that there is very little of this enzyme present in the species as a whole. Examination of other gorilla placentae should establish which of these possibilities is correct.

By comparing the specific activity of pure placental phosphatase with that of 150,000g max pellets of placental extracts, we have estimated that on average, 1% of the membrane protein of human placentae is alkaline phosphatase. The very low activity in rhesus, African green, baboon and spider monkeys and possibly gorillas, and the apparent biochemical distinction of the enzymes in these species is extremely interesting. No other human tissue has been found to have an enzyme which does not occur in the corresponding tissue in monkeys. The presence of this enzyme in such high concentrations in the human placenta is therefore species specific. This may account for the unusually high degree of heterozygosity at this locus⁷ and the apparent role of several genetic variants in prenatal selection^{14,15}. If the expression of the enzyme during human fetal development is a late evolutionary event, then selection of phenotypes could still be much more active than that of other enzymes whose organ-specific function has become specialised over a longer period of evolution.

The similarity of orangutan, chimpanzee and human placental phosphatase by double diffusion is interesting. It suggests that, despite the occurrence of this enzyme late in primate evolution, the gene coding for it is similar in all three species. It is likely that an enzyme similar to human, chimpanzee and orangutan placental alkaline phosphate is normally present in an organ(s) other than the placenta (or at much lower concentrations in the

placenta), and that its presence in the placenta is the species-specific event. This could have occurred after a gene duplication or by de-repression of a regulator gene in the placenta¹⁶.

The production of significant amounts of placental-type alkaline phosphatase, the so-called 'Regan isoenzyme'¹⁷, and variants of this enzyme^{18,19} in some cancer patients, has been interpreted as being due to de-repression of a fetus-specific gene product²⁰. If the gene coding for placental phosphatase is in fact expressed in some other as yet unidentified organ, then the occurrence of the enzyme in this organ pre-dates its occurrence in the placenta. The production of placental-type alkaline phosphatase by the placenta and certain tumours may therefore be a coincidence, not the expression of fetal genes in neoplasia. This hypothesis might be tested by investigating the presence of placental-type alkaline phosphatase in non-neoplastic human tissues and studying these same tissues in non-human primates.

In view of the apparent species specificity of the placental phosphatase, it would be interesting to develop a complete catalogue of the occurrence of this enzyme type in non-human primates. It is possible that this might reveal a new biochemical aspect of primate evolution, especially if the function of this enzyme is determined.

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1. Fishman, W. H. *Am. J. Med.* **56**, 617-650 (1974).
2. Boyer, S. H. *Ann. N.Y. Acad. Sci.* **103**, 938-950 (1963).
3. Sussman, H. H., Small, P. A. & Cotlove, E. *J. Biol. Chem.* **243**, 160-166 (1968).
4. Robson, E. B. & Harris, H. *Ann. hum. Genet.* **30**, 219-232 (1967).
5. Beckman, L., Beckman, G., Christodoulou, C. & Ifekwumigwe, A. *Acta genet., Basel* **17**, 406-412 (1967).
6. Donald, L. J. & Robson, E. B. *Ann. hum. Genet.* **37**, 303-313 (1974).
7. Harris, H. H., Hopkinson, D. A. & Robson, E. B. *Ann. hum. Genet.* **37**, 237-253 (1974).
8. Manning, J. P., Inglis, N. R., Green, S. & Fishman, W. H. *Enzymologia* **37**, 251-261 (1969).
9. VanBelle, H., DeBroe, M. E. & Wieme, R. J. *clin. Chem.* **23**, 454-459 (1977).
10. Doellgast, G. J. & Fishman, W. H. *Clin. chim. Acta* **75**, 449-454 (1976).
11. Doellgast, G. J., Guenther, R. A., Spiegel, J. & Fishman, W. H. *Biochim. biophys. Acta* **484**, 59-78 (1977).
12. Walker, A. *Molecular Anthropology*, 63-77 (Plenum, New York, 1976).
13. Donald, L. J. & Robson, E. B. *Ann. hum. Genet.* **38**, 7-18 (1974).
14. Beckman, G., Beckman, L. & Magnusson, S. S. *Hum. Hered.* **22**, 473-480 (1972).
15. Beckman, G. & Beckman, L. *Hereditas* **81**, 85-88 (1975).
16. Zuckerkandl, E. *Molecular Anthropology*, 387-447 (Plenum, New York, 1976).
17. Fishman, W. H. *et al. Nature* **219**, 697 (1968).
18. Nakayama, T., Yoshida, M. & Kitamura, M. *Clin. chim. Acta* **30**, 546-548 (1970).
19. Higashio, K., Hashinotsume, M., Kong, K. Y., Takahashi, Y. & Yamamura, Y. *Clin. chim. Acta* **40**, 67-81 (1972).
20. Fishman, W. H. & Singer, R. M. *Cancer*, 57-80 (Plenum, New York, 1976).

Human placental alkaline phosphatase differs from that of other species

AT LEAST three gene loci seem to be involved in determining the various forms of human alkaline phosphatase (ALP)—one coding for the placental form of the enzyme, at least one coding for the intestinal forms, and at least one for the liver, bone and kidney forms^{1,2}. These three classes of human alkaline phosphatase can be distinguished from one another by their

Table 1 Concentrations of various inhibitors required to produce 50% inhibition of alkaline phosphatase in placenta and liver of various mammals

		[I] ₅₀ (mM)		
		HA	Phe	PGG
Man	Placenta	62.88	1.13	0.09
	Liver	2.83	28.62	27.44
Dog	Placenta	2.87	28.42	32.52
	Liver	2.91	22.42	27.44
Mouse	Placenta	2.73	49.08	36.30
	Liver	2.13	28.26	28.13
Rat	Placenta	2.99	42.94	29.78
	Liver	2.99	26.75	35.98
Sheep	Placenta	2.63	45.82	30.95
	Liver	2.66	30.58	32.93
Cow	Placenta	2.59	30.10	26.05
	Liver	2.83	37.61	27.40
Pig	Placenta	2.82	25.83	30.19
	Liver	2.79	39.51	38.45
Guinea pig	Placenta	2.35	33.41	26.98
	Liver	3.80	27.77	33.36
Rhesus	Placenta	2.90	40.28	38.94
	Liver	3.28	27.00	39.12
Hamster	Placenta	2.77	33.35	34.12
	Liver	2.44	38.32	34.86
Cat	Placenta	2.42	29.92	27.16
	Liver	2.41	33.73	29.95

The tissues were kept frozen at -20°C until extracted. Aqueous solutions of the enzyme were obtained by extracting the tissue in the presence of *n*-butanol as previously described¹. Assays were carried out using *p*-nitrophenyl phosphate (5.0 mM) as substrate in a diethanolamine buffer 1.0 M pH 9.85, containing 0.28 M NaCl and 0.5 mM MgCl_2 . The inhibitors were added to the reaction mixture to produce final concentrations of 1, 2.5, 5, 10 and 20 mM. In the case of human placenta with PGG, assays were also carried out in the presence of 0.2 and 0.5 mM of this inhibitor. The reactions were run for up to 30 min at 30°C and stopped by the addition of a volume of 1 M phosphate buffer, pH 9.8, equal to the volume of the reaction mixture. Absorbance was read at 405 nm. Degree of inhibition was expressed as percentage of the original activity remaining in the presence of a given concentration of inhibitor. Two or more determinations were made on each sample at each inhibitor concentration and in some cases samples from different individuals of the same species were examined. The average value was used in the subsequent calculations. Estimates of $[I]_{50}$ were obtained by calculating the least-squares regression of (100/per cent activity remaining) on concentration of inhibitor, $[I]$, and determining from this the concentration of inhibitor required to give 50% inhibition (see Fig. 1 legend).

behaviour with certain inhibitors, their thermostabilities and their electrophoretic and immunological characteristics. Recent studies have shown that in the dog, the ALP present in placenta closely resembles that in liver, and that these enzymes are very similar to the ALP present in human liver but different from the ALP present in human placenta^{3,4}. It has also been reported that human placental ALP differs from other mammalian placental ALPs in certain respects (for example, sensitivity to L-phenylalanine inhibition)^{5,6}. In view of these results we have examined some of the characteristics known to distinguish human placental and human liver ALPs, in the liver and placental ALPs from a series of mammalian species. The species from which both tissues were obtained included rodents, carnivores, ungulates and one primate (rhesus). We report here that the properties of human placental ALP differ from those of human liver ALP, whereas in the other species studied, placental and liver ALPs show similar properties.

Particularly sharp discrimination between human liver and placental ALPs can be achieved using L-homoarginine (HA), L-phenylalanine (Phe) and L-phenylalanyl-glycylglycine (PGG) as inhibitors¹. HA produces marked inhibition of liver ALP at concentrations which produce only slight inhibition of placental ALP⁷. In contrast, Phe and PGG produce marked inhibition of placental ALP at concentrations which produce only slight inhibition of liver ALP⁸. This combination of inhibitors also distinguishes intestinal ALP from both liver and placental ALP^{1,9}. The percentage activity remaining in the presence of various concentrations (1–20 mM) of each of these inhibitors was determined for the different liver and placental ALPs of the various animal species. As a general rule the reciprocal of the reaction velocity of an enzyme may be expected to vary linearly

with the concentration of an inhibitor^{10,11}. This was found to be the case for the various ALPs with these particular inhibitors. Some typical plots are shown in Fig. 1. The greater the slope of the regression line, the greater the degree of inhibition produced by the particular inhibitor. The least-squares regression was therefore calculated for each set of data and from this the concentration of inhibitor required to produce 50% inhibition of the enzyme, $[I]_{50}$, was estimated. These estimates are given in Table 1 for the liver and placental ALPs of each species with each of the inhibitors.

It is apparent that for each of the inhibitors the estimated $[I]_{50}$ for human placenta is quite different from the estimated $[I]_{50}$ values for any of the placentas from the other species. Table 2 gives the means of the $[I]_{50}$ estimates for the series of non-human placental ALPs and for the liver ALPs. They are in close agreement. A paired *t*-test gives no evidence for significant differences between $[I]_{50}$ placental ALP and $[I]_{50}$ liver ALP in any of the species (HA, $t = 0.68$, d.f. = 9, $P > 0.5$; Phe, $t = 1.28$, d.f. = 9, $P > 0.2$; PGG, $t = 0.91$, d.f. = 9, $P > 0.2$). Note that the $[I]_{50}$ values for human liver ALP for each of the inhibitors do not deviate significantly from the mean $[I]_{50}$ values for placenta or liver ALPs of the other species. In contrast, the $[I]_{50}$ for human placental ALP with HA is about 30 times greater than the mean $[I]_{50}$ for the liver and placental ALPs of the other species; and the mean $[I]_{50}$ values for the liver and placental ALPs of the other species with Phe and PGG are about 30 times and 300 times greater, respectively, than the corresponding $[I]_{50}$ values for human placental ALP. An analysis of variance of the placental and liver ALP $[I]_{50}$ values was carried out to see whether there were significant differences between the non-human species. However, no significant heterogeneity was detected (HA, $F = 1.03$, d.f. = 9, 10, $P > 0.25$; Phe, $F = 0.41$, d.f. = 9, 10, $P > 0.75$; PGG, $F = 1.91$, d.f. = 9, 10, $P > 0.10$).

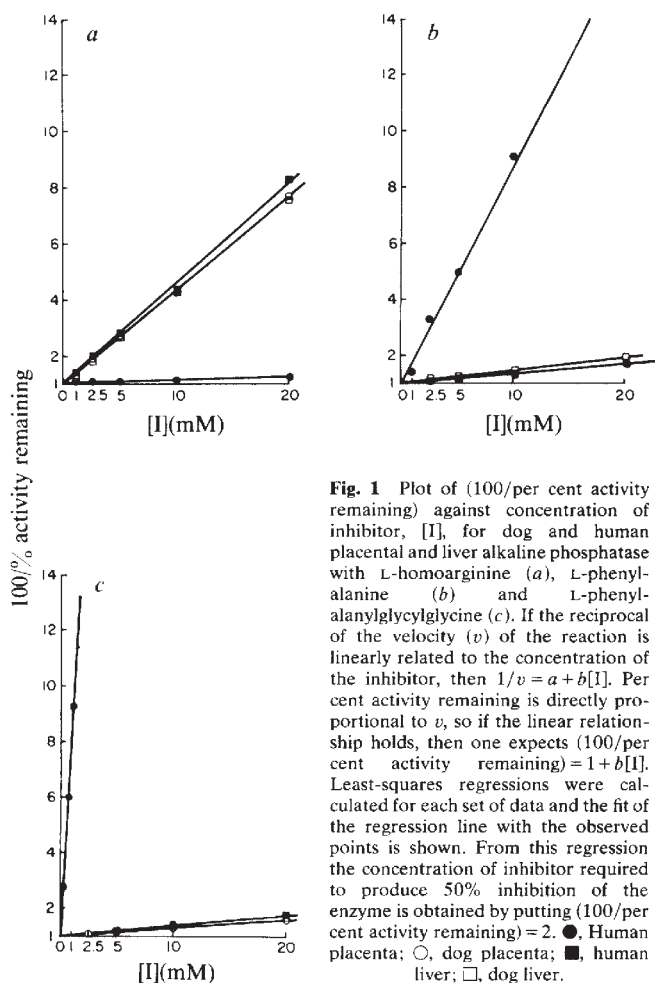


Fig. 1 Plot of (100/per cent activity remaining) against concentration of inhibitor, $[I]$, for dog and human placental and liver alkaline phosphatase with L-homoarginine (a), L-phenylalanine (b) and L-phenylalanyl-glycylglycine (c). If the reciprocal of the velocity (v) of the reaction is linearly related to the concentration of the inhibitor, then $1/v = a + b[I]$. Per cent activity remaining is directly proportional to v , so if the linear relationship holds, then one expects (100/per cent activity remaining) = $1 + b[I]$. Least-squares regressions were calculated for each set of data and the fit of the regression line with the observed points is shown. From this regression the concentration of inhibitor required to produce 50% inhibition of the enzyme is obtained by putting (100/per cent activity remaining) = 2. ●, Human placenta; ○, dog placenta; ■, human liver; □, dog liver.

Thus, the data suggest that the structures of the binding site(s) for HA, Phe and PGG must be identical or at least very similar in the placental and liver ALPs of these various species and also in human liver ALP. However, the structure of the binding site(s) for these inhibitors in human placental ALP is presumably quite different.

Table 2 Means and standard deviations of estimated $[I]_{50}$ values for various inhibitors for human and non-human placental and liver alkaline phosphatase

			[I] ₅₀ (mM)					
		No. of species	HA		Phe		PGG	
			Mean	s.d.	Mean	s.d.	Mean	s.d.
Man	Placenta	1	62.88	—	1.13	—	0.09	—
	Liver	1	2.83	—	28.62	—	27.44	—
Mammals (excluding man)	Placenta	10	2.71	0.21	35.92	8.03	31.30	4.21
	Liver	10	2.82	0.47	31.20	5.80	32.76	4.40

$[I]_{50}$ values for HA, Phe and PGG are from 10 different mammalian species compared with the corresponding values for the human enzymes.

Human placental ALP is remarkably thermostable¹². It may be heated at 65 °C for more than 1 h with little or no loss of activity. In contrast, human liver ALP is rapidly destroyed in these conditions. The thermostabilities of the various animal liver and placental ALPs were therefore determined. Each of the samples was heated at 65 °C and also at 56 °C for different times up to 30 min and the residual activities determined. In each case the regression of the (log percentage activity remaining) on time was calculated, and from this an estimate of the time required to produce 50% inactivation of the enzyme, T_{50} , was obtained. The results (Table 3) indicate that the various non-human placental and liver ALPs are relatively thermostable and indeed similar to human liver ALP in this respect. The mean T_{50} values for placental and liver ALPs of the non-human species

Table 3 Time required to produce 50% inactivation of alkaline phosphatase in placenta and liver of various mammals

		56 °C T_{50} (min)	65 °C T_{50} (min)
Man	Placenta	Stable	Stable
	Liver	8.2	1.05
Dog	Placenta	23.1	1.20
	Liver	14.2	1.31
Mouse	Placenta	2.3	0.58
	Liver	5.8	0.80
Rat	Placenta	2.2	0.42
	Liver	5.0	0.84
Sheep	Placenta	10.8	0.52
	Liver	9.0	0.35
Cow	Placenta	10.9	1.04
	Liver	16.0	1.32
Pig	Placenta	9.7	0.91
	Liver	3.5	0.65
Guinea pig	Placenta	6.6	0.97
	Liver	6.6	0.92
Rhesus	Placenta	8.2	0.76
	Liver	18.7	0.45
Hamster	Placenta	4.4	0.21
	Liver	7.9	0.32
Cat	Placenta	12.3	0.67
	Liver	18.2	0.60

Thermal inactivations were carried out at 56 °C and 65 °C on tissue extracts dialysed against 10.0 mM Tris-HCl, pH 7.5, containing 2 mM MgCl₂. The samples (0.2 ml) were placed in capped plastic tubes (Falcon 2038) and heated in a waterbath for 5, 10, 15, 20 or 30 min at 56 °C and for 1, 2.5, 5, 10, 15 or 20 min at 65 °C. At the end of each period the tube was immediately placed in an ice bath where an unheated control had been kept for the same period. Thermal inactivation was expressed as percentage of original activity remaining after a given time at the particular temperature. Two or more determinations were made on each sample at each period and in some cases samples from different individuals of the same species were examined. The average values were used in subsequent calculations. Estimates of T_{50} were obtained by calculating the least-squares regression of (log per cent activity remaining) on time and determining from this the time required for 50% inactivation.

(Table 4) are in close agreement. A paired *t*-test showed no evidence for significant differences between the placental or liver ALP T_{50} values in any of the species. The human liver ALP T_{50} value does not deviate significantly from either the placental or liver ALP mean T_{50} values. Human placental ALP is, of course, quite stable in these conditions. An analysis of variance of the T_{50} values for the non-human placental and liver ALPs was carried out to see whether there was evidence for significant differences between the species. Significant heterogeneity was indeed detected (56 °C, $F = 3.29$, d.f. = 9, 10, $0.01 < P < 0.05$; 65 °C, $F = 7.29$, d.f. = 9, 10, $P < 0.01$). Such thermostability differences presumably reflect differences in the overall molecular structures of the ALPs in the different species.

Table 4 Means and standard deviations of estimated T_{50} values for human and non-human placental and liver alkaline phosphatase

		56 °C T_{50} (min)		65 °C T_{50} (min)	
Man	Placenta	Stable	—	Stable	—
	Liver	8.2	—	1.05	—
Mammals (excluding man)	Placenta	9.1	6.1	0.73	0.31
	Liver	10.5	5.7	0.76	0.36

The T_{50} values at 56 °C and 65 °C for placental and liver alkaline phosphatases were from 10 different mammalian species compared with the corresponding values for the human enzymes.

The results show that in terms of the inhibition and thermostability characteristics studied here, placental and liver ALPs are indistinguishable in each of these mammalian species other than man. The data suggest that the same gene locus is being expressed in liver and placenta of the various species and that this locus corresponds to the ALP locus being expressed in human liver. It is, however, presumably different from the ALP locus expressed in human placenta.

These findings are of evolutionary interest. It has been suggested that the liver/bone/kidney, intestinal, and in man, placental, ALPs represent a so-called multilocus enzyme system, the loci coding for these enzymes having been derived in the course of evolution from a common ancestral gene as a result of successive gene duplications^{4,13}. Mutations occurring in the genes subsequent to the duplications would have led to some divergence in their base sequences, and thus to characteristic differences in the properties of the ALPs they now determine. If this is correct, a plausible interpretation of the data is that the gene duplication giving rise to the human placental ALP gene locus was a relatively late evolutionary event which occurred subsequent to the divergence of the evolutionary lineage leading to man from the various lineages leading to most other mammalian species, including rhesus, an Old World monkey. An alternative hypothesis would be that a gene locus corresponding to the human placental ALP locus does exist in these other mammalian species but is not expressed in their placentas, so that the late evolutionary event would have involved its de-repression or activation accompanied by repression of the locus coding for liver ALP. This seems a less economical hypothesis. However, eventually it should be possible to distinguish critically between the two hypotheses, by direct studies of the genes at the DNA level using the newly emerging methods of DNA analysis, such as restriction endonuclease mapping. The apparently late evolutionary appearance of the human placental ALP locus is also of interest in connection with the fact that human placental ALP, unlike the intestinal and liver ALPs, is genetically highly polymorphic.

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- Mulivor, R. A., Plotkin, L. I. & Harris, H. *Ann. hum. Genet.* **42**, 1-13 (1978).
- Mulivor, R. A., Hannig, V. L. & Harris, H. *Proc. natn. Acad. Sci. U.S.A.* **75**, 3909-3912 (1978).
- Van Belle, H. *Biochim. biophys. Acta* **289**, 158-168 (1972).
- Moak, G. & Harris, H. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1948-1951 (1979).
- Manning, J. P., Inglis, N. R., Green, S. & Fishman, W. H. *Enzymologia* **37**, 251-261 (1969).
- Manning, J. P., Inglis, N. R., Green, S. & Fishman, W. H. *Enzymologia* **39**, 307-318 (1970).
- Lin, Ch.-W. & Fishman, W. H. *J. biol. Chem.* **247**, 3082-3087 (1972).
- Fishman, W. H. & Sie, H.-G. *Enzymologia* **41**, 141-167 (1971).
- Doellgast, G. J. & Fishman, W. H. *Clin. chim. Acta* **75**, 449-454 (1977).
- Dixon, M. *Biochem. J.* **55**, 170-171 (1953).
- Bergmeyer, H. U. & Gawehn, K. *Principles of Enzymatic Analysis* (Chemie, Weinheim, 1978).
- Neale, F. C., Clubb, J. S., Hotchkis, D. & Posen, S. *J. clin. Path.* **18**, 359-363 (1965).
- Harris, H. *CIBA Fdn Symp.* **66** (New Ser.) (in the press).

α -Globin gene organisation in blacks precludes the severe form of α -thalassaemia

In most human populations, the α -globin structural gene loci are duplicated so that each diploid cell contains four copies of α -globin genes¹⁻³. In α -thalassaemia, a hereditary disorder of α -globin chain synthesis, the most common molecular lesion is due to the deletion of the α -globin genes⁴⁻⁷. In the Asian population, four main α -thalassaemia syndromes of increasing clinical severity are recognised: (1) the silent carrier state (α -thalassaemia-2) with no clinical manifestation; (2) α -thalassaemia trait (α -thalassaemia-1), characterised by microcytic red blood cells but little or no anaemia; (3) haemoglobin-H disease, which manifests as haemolytic anaemia; and (4) homozygous α -thalassaemia, in which the afflicted fetus dies at or around term from hydrops fetalis. These four syndromes are due to the deletion of from one to all four copies of the α -globin genes. In this study, we have characterised α -thalassaemia in people of African origin. Haemoglobin screening programmes have shown that α -thalassaemia occurs in the black population⁸⁻¹⁰. Recently we have demonstrated by complementary DNA-DNA hybridisation that in black individuals with clinically well defined α -thalassaemia trait, two of the four normal α -globin genes were deleted¹¹. However, in this population, haemoglobin-H disease is rare and homozygous α -thalassaemia has never been found^{12,13}. For this study we have used the restriction endonuclease mapping technique of Southern¹⁴ to delineate the nature of the deletion of the α -globin genes.

The two α -globin structural gene loci are 3.7 kilobases apart^{15,16}. The presence of these two loci could be deduced by digestion with the two restriction enzymes, *EcoRI* and *HpaI*. When cellular DNA from a non-thalassaemic individual (genotype: $\alpha\alpha/\alpha\alpha$) was digested with *EcoRI*, the two α -globin loci were located in a single DNA fragment about 23 kilobases in length. In the DNA of a patient with haemoglobin-H disease who had only one α -globin gene remaining ($-\alpha/-\alpha$), the *EcoRI* fragment which contained the single α -globin locus became shortened to 19 kilobases. In the silent carrier state (α -thalassaemia-2), in which two α -globin loci resided on one chromosome and a single locus resided on the other ($-\alpha/\alpha\alpha$), both the 23-kilobase and the 19-kilobase bands were found. The phenotype of α -thalassaemia trait with two α -globin genes deleted could arise from two types of gene arrangement. The

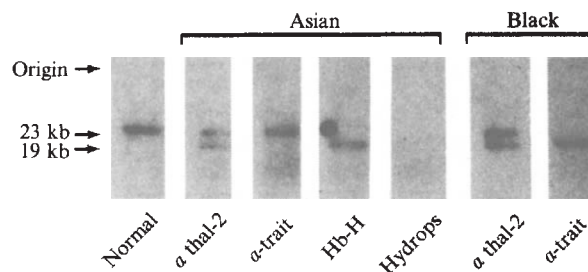


Fig. 1 *EcoRI*-digested human DNA hybridised with α -globin gene specific probe. Human DNA was extracted from white blood cells or placenta from normal individuals and patients with different α -thalassaemia syndromes using methods described previously⁵. DNA (10 μ g) digested with *EcoRI* was separated by electrophoresis on 0.8% agarose gel and transferred on to nitrocellulose filters as described previously¹⁷. The filter was hybridised for 48 h in previously described conditions¹⁷ with plasmid JW 101 (ref. 18) which contains the human α -globin complementary DNA insert and which has been nick translated and labelled with ³²P-dCTP and ³²P-dTTP according to the method of Maniatis *et al.*¹⁹. The filter was extensively washed and autoradiographed¹⁷. The numbers on the left indicate nucleotide pairs in kilobases (kb). The α -globin gene containing bands in Asians are shown on the left, whereas those of blacks are on the right. Note the difference in the patterns of α -thalassaemia trait (α -trait) in the two ethnic groups.

two genes could be deleted either *in cis* or *in trans*, giving rise to heterozygous state of α -thalassaemia-1 ($-\alpha/\alpha$) or the homozygous state of α -thalassaemia-2 ($-\alpha/-\alpha$), respectively. These two gene arrangements could be distinguished, as *EcoRI* digests would show a single 23-kilobase normal fragment in the former, and a single 19-kilobase fragment in the latter (Fig. 1).

The arrangement of the α -globin genes could further be confirmed by digestion of the DNA with the enzyme *HpaI*, which cleaves in between the two α -globin loci (Fig. 2). Normal DNA yielded two *HpaI* fragments, 14.5 and 4.1 kilobases in length, which contained the 3' and the 5' α -globin loci, respectively^{15,16}. In haemoglobin-H disease the single remaining locus contained in the 14.5-kilobase fragment was seen. In the silent carrier state both the 14.5-kilobase and the 4.1-kilobase fragments were present, the former containing two alleles, being

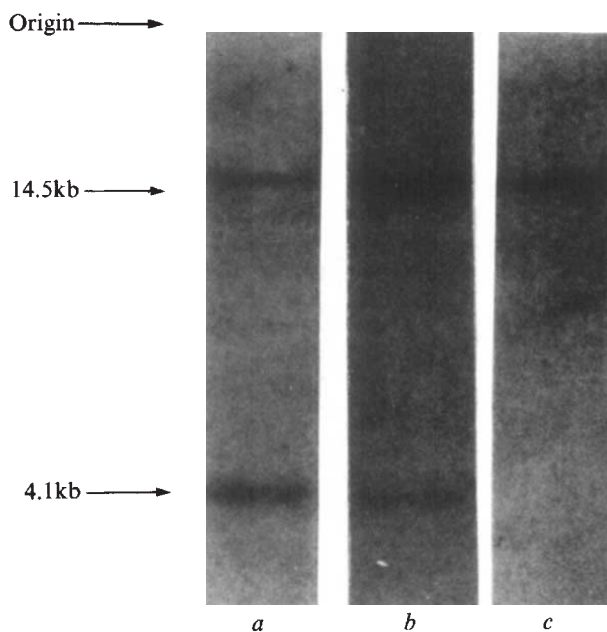


Fig. 2 *HpaI* digested human DNA hybridised with α -globin gene-specific probe. The pattern seen in: a, normal ($\alpha\alpha/\alpha\alpha$) and α -thalassaemia-1 with deletion *in cis* ($-\alpha/\alpha$); b, α -thalassaemia-2 ($-\alpha/\alpha$); c, haemoglobin-H disease ($-\alpha/-\alpha$) or α -thalassaemia trait with deletion *in trans* ($-\alpha/-\alpha$).

Table 1 Pertinent haematological data of patients with α -thalassaemia trait

	Mean corpuscular volume	Hb A ₂ (%) ²	Hb Bart's (%)	Serum iron (μ g per 100 ml)	Free erythrocyte protoporphyrin (μ g per 100 ml RBC)	α/β Ratio	α -Globin [†] gene no.
<i>Normal</i>							
Adult	80–99	1.5–3.0	—	65–165	<90	0.95–1.05	4
Cord blood	≥ 95	—	trace	—	—	—	4
<i>Black patients</i>							
Adult (9)	72 (64–75)	2.2 (1.9–2.7)	—	82 (68–130)	35 (23–70)	0.76 (0.62–0.87)	2
Cord blood (6)	92* (88–95)	—	3.1 (3.0–5.0)	70† (64–120)	—	—	2
<i>Asian patients</i>							
Adult (15)	70 (62–75)	2.3 (1.8–2.9)	—	98 (65–162)	46 (29–85)	0.82 (0.71–0.93)	2

* Follow-up studies showed persistent microcytosis at 6–12 months.

† Determined at 6–12 months.

‡ Determined by complementary DNA–DNA hybridisation.

more intense than the latter with one allele. Again, the two genotypes which produce the clinical state of α -thalassaemia trait could be distinguished from each other, as deletion *in cis* ($-\alpha/-\alpha$) gave both the 14.5 and 4.1-kilobase bands, whereas deletion *in trans* ($-\alpha/-\alpha$) produced only the 14.5-kilobase band.

We selected 15 black individuals with clinical diagnosis of α -thalassaemia trait. Rigid criteria were used to establish the diagnosis. These were: in the adult, a mean corpuscular volume (MCV) of less than 76 fl, normal haemoglobin A₂ and F, absence of iron deficiency and decreased α/β globin synthesis ratio; in the newborn period, an MCV of less than 96 fl, haemoglobin Bart's of over 3% and persistence of microcytosis at the age of 6–12 months in the absence of iron deficiency. Table 1 summarises the pertinent haematological findings. Haemoglobin-H was not detectable in these patients and molecular hybridisation analysis showed that they had two copies of α -globin structural genes per diploid genome¹¹. Restriction endonuclease mapping showed that all 15 had the 19-kilobase band on *Eco*RI digest, and only the 14.5-kilobase band on *Hpa*I digest. Thus, the two remaining α -globin genes are located on opposite chromosomes ($-\alpha/-\alpha$) (Fig. 1). We also studied 15 adult Asian patients with α -thalassaemia trait. This diagnosis was also made according to the criteria listed, and in addition, six of these patients were parents of hydropic fetuses. Molecular hybridisation confirmed

that they had two α -globin genes per diploid genome¹¹. Restriction analysis showed that they all had the normal 23-kilobase *Eco*RI band and both *Hpa*I bands, indicating that the deletion of the two genes had occurred on the one chromosome ($-\alpha/-\alpha$).

We used these enzymes to map the α -globin genes in 211 black Americans. They were not selected for α -thalassaemia, none had haemoglobin-H disease, but some had the β -structural mutants S and C (Table 2); 70.6% had the normal α -globin genotype, 27.5% had two α -globin bands on *Eco*RI, characteristic of the silent carrier state, and 1.9% had the 19-kilobase *Eco*RI band and only the 14.5-kilobase *Hpa*I band characteristic of the genotype of $-\alpha/-\alpha$. The frequency of the single α -globin locus ($-\alpha$) was calculated to be 0.16.

We also investigated the mechanism of transmission of α -thalassaemia. Previously, 'direct transmission' of α -thalassaemia trait from parent to offspring in the black has been taken as evidence for the presence of the α -thalassaemia-1 genotype ($-\alpha/\alpha$). When we studied with restriction enzyme analyses two families in which direct transmission seemed to have occurred, we found that the parent with α -thalassaemia trait had the genotype of homozygous α -thalassaemia-2 (that is, deletion of the two α -globin genes on opposite chromosomes), and the haematologically normal spouse had the 23- and 19-kilobase bands containing the α -globin genes characteristic of α -thalassaemia-2 carrier. The latter diagnosis was previously unrecognised without restriction endonuclease mapping. Figure 3 illustrates the findings in one such family. As the frequency of α -thalassaemia-2 is 27.5%, this type of inheritance will explain the so-called direct transmission in most cases.

These findings directly confirm the hypothesis first proposed by Lehmann on the genetic basis of α -thalassaemia and the lack of the severe form of α -thalassaemia in the black population²⁰. Using the percentages of haemoglobin S in sickle-cell trait as an indicator of α -thalassaemia, Altay *et al.*⁹ in the African population and Brittenham²¹ in Asian Indians found a trimodal distribution of the percentage of haemoglobin S level, of about, 25, 33 and 45%. They concluded that the two lower percentages of S are due to the co-existence of α -thalassaemia trait and α -thalassaemia-2, respectively. The present study directly demonstrates the frequent occurrence of the α -thalassaemia-2 genotype. It seems that the presence of 1–2% haemoglobin Bart's in cord blood used as the criterion for diagnosis of α -thalassaemia-2 grossly underestimates the presence of the α -thalassaemia-2 gene, as electrophoresis of cord blood showed that only a few per cent of the black population had increased haemoglobin Bart's, whereas restriction enzyme analysis demonstrated the prevalence of α -thalassaemia-2 carrier to be 27.5%. This finding agrees with that of Pierce *et al.*¹⁰ who detected a high incidence of α -thalassaemia in black recruits by globin chain synthesis studies.

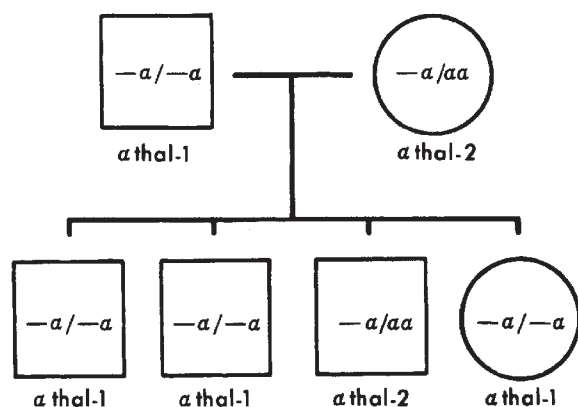


Fig. 3 Inheritance of α -thalassaemia in a black family. The mean corpuscular volume in femtolitres (normal > 80) in the family members are: I-1: 70, I-2: 81, II-2: 68, II-3: 78 (age 4 yr, normal > 75), III-4: 62. The apparent direct transmission of α -thalassaemia trait is due to the presence of α -thalassaemia-2 in the haematologically normal mother.

Table 2 α -globin genotype in black Americans

Genotype	No.	Percentage
$\alpha\alpha/\alpha\alpha$	149	70.6
$-\alpha/\alpha\alpha$	58	27.5
$-\alpha/-\alpha$	4	1.9
Total	211	100

Frequency of $(-\alpha)$ genotype = 0.16.

These results satisfactorily explain the distribution of the different clinical syndromes of α -thalassaemia in the black population. Whereas α -thalassaemia-1 with deletion of the two α -globin genes *in cis* ($---/\alpha\alpha$) is common in the Asian, this genotype is rare in the black. The fatal form of α -thalassaemia associated with deletion of all the α -globin genes ($---/---$) will therefore not be found. Haemoglobin H disease ($---/\alpha$) will also be rare as it requires the combination of an α -thalassaemia-1 gene with an α -thalassaemia-2 gene. On the other hand, in those parts of the world such as the West Indies, where there is a high incidence of mating between people of African and Asian origin, a high incidence of haemoglobin-H is found.

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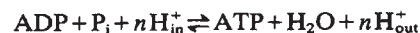
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- Lehmann, H. & Carrell, R. W. *Br. med. J.* **4**, 748–750 (1968).
- Hollan, S. R. *et al. Nature* **235**, 47–50 (1972).
- Lie-Injo, L. E., Ganesan, J., Clegg, J. B. & Weatherall, D. J. *Blood* **43**, 251–259 (1974).
- Ottolenghi, S. *et al. Nature* **251**, 389–392 (1974).
- Taylor, J. M. *et al. Nature* **251**, 392–393 (1974).
- Kan, Y. W. *et al. Nature* **255**, 255–256 (1975).
- Ramirez, F. *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 1550–1554 (1975).
- Schwartz, E. & Atwater, J. J. *clin. Invest.* **51**, 412–418 (1972).
- Altay, C. *et al. Pediat. Res.* **11**, 147–152 (1977).
- Pierce, H. I., Kurachi, S., Sofroniadou, K. & Stamatoyannopoulos, G. *Blood* **49**, 981–986 (1977).
- Davis, J. R. Jr *et al. Am. J. hum. Genet.* (in the press).
- Esan, G. J. F. *Br. J. Haemat.* **19**, 47–56 (1970).
- Stamatoyannopoulos, G., Haywood, D. & Papayannopoulos, T. *Birth Defects Original Article Series* **8m**, 29–38 (1972).
- Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
- Orkin, S. H. *et al. Cell* **17**, 33–42 (1979).
- Embury, S. H., Lebo, R. V., Dozy, A. M. & Kan, Y. W. *J. clin. Invest.* **63**, 1307–1310 (1979).
- Kan, Y. W. & Dozy, A. M. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5631–5635 (1978).
- Wilson, J. T. *et al. Nucleic Acids Res.* **5**, 563–581 (1978).
- Maniatis, T., Jeffrey, A. & Kleid, D. G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1184–1188 (1975).
- Lehmann, H. *Lancet* **ii**, 78–80 (1970).
- Brittenham, G. *Nature* **268**, 635–636 (1977).

Phosphorylation in a simple system of lipids and chloroplast ATP synthetase driven by pulsed ionising radiation

WE report here the observation of an ADP-dependent phosphorylation reaction in an artificial lipid vesicle system driven by pulsed ionising radiation. This reaction is completely dependent on the only enzyme incorporated into the phospholipid vesicle bilayer, the ATP synthetase (CF_0 – CF_1) complex from spinach chloroplasts. Previous attempts to demonstrate directly an obligatory coupling between the proton pumping and phosphorylation activities of an ATP synthetase enzyme in resolved, reconstituted vesicle systems have required the presence of additional protein components^{1–3} to generate the transmembrane pH gradient and/or membrane potential postulated by the chemiosmotic hypothesis⁴ as the driving force for the ATP synthesis reaction:



Pulsed high-energy radiolysis of water produces, in a very brief time increment, significant quantities of protons along with the highly reactive transient species e_{aq}^- , $OH^\cdot$, and $H^\cdot$ ($\Delta[H^\cdot] \sim 10^{-5}$ M in 50 ns)⁵. Assuming a uniform production of protons throughout the aqueous media, differential buffering between the inner and outer aqueous compartments of phospholipid vesicles will result in the effectively instantaneous formation of a transmembrane proton gradient. We have applied the pulse radiolysis technique to drive the ATP synthetase activity of phospholipid vesicles reconstituted with the chloroplast CF_0 – CF_1 enzyme complex.

Single-walled bilayer vesicles were prepared in dilute buffer from purified soy phospholipids (asolectin) by either a sonication⁶ or a detergent-dilution⁷ technique. The CF_0 – CF_1 ATP synthetase complex extracted from spinach was incorporated into the vesicles as previously described⁸. Immediately before irradiation, a highly buffered solution containing ADP and $^{32}P_i$ was added; then the sample was deoxygenated with nitrogen, sealed in a small glass vial and irradiated. Details of the specific reaction mixtures for each experiment and the method of assay for esterified $^{32}P_i$ are given in the footnotes to the tables. The radiation dose given per pulse was determined by a method previously described⁹.

From Table 1 it is apparent that phosphorylation only occurred in irradiated samples, and that the yield of phosphorylated product reached a limiting value at high cumulative dose levels. This is consistent with the predicted radiation inactivation of the enzyme; based on studies with rat-liver mitochondria, inactivation would be expected in the 10^2 krad range¹⁰. Significantly, the amount of $^{32}P_i$ esterified exceeded, on a molar basis, the amount of enzyme added by a factor of about 8 in the cholate-dilution vesicles and by a factor of about 2 in sonicated vesicles. Even assuming that all of the enzyme added was active and properly

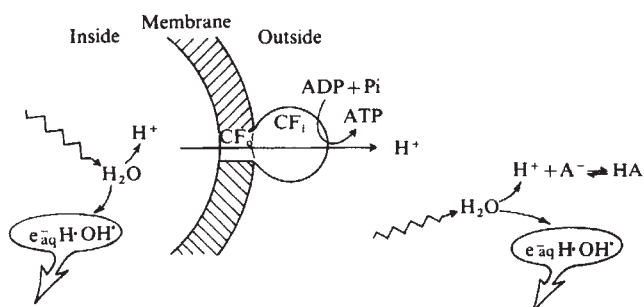


Fig. 1. Proposed mechanism for radiolytically driven ATP formation in artificial lipid vesicles reconstituted with the chloroplast CF_0 – CF_1 complex.

Table 1 Dose dependence for pulse radiolysis-driven phosphorylation in artificial lipid vesicles

No. of pulses*	Pulse frequency (Hz)	$^{32}\text{P}_i$ esterified (nmol ml $^{-1}$)†	
		Cholate-dilution vesicles‡	Sonicated vesicles§
0	—	0	0
100	120	0.33	
500	120		1.46
1,000	5	0.92	1.55
1,000	120	0.95	1.63
10,000	120	0.97	

*Radiation dose in each pulse, 1 krad. Pulse width, 50 ns.

†Residual radioactivity after extraction of unreacted ^{32}P -orthophosphate as phosphomolybdic acid as described^{3,12} $^{32}\text{P}_i$ specific activity, 3,375 c.p.m. nmol $^{-1}$.

‡Cholate-dilution vesicles were prepared as follows: 20 mg purified soy phospholipid was dissolved in 0.5 ml of a solution containing 0.2 M sucrose, 1.4% Na-cholate, 1 mM tricine (pH 8.0) and 3 mM MgCl₂. This was mixed with an equal volume of CF₀-CF₁ complex containing 8.0 mg protein per ml. After 20 min at 4 °C, 16- μ l aliquots of the enzyme-lipid mixture were diluted into 0.8 ml volumes of a solution containing 0.8 mM tricine (pH 8.0), 3 mM MgCl₂, 12 mM glucose, 0.12 mM KNO₃. After 1–2 min, 0.1 ml 10% defatted bovine serum albumin and 0.1 ml of the external buffer solution (2 mM ADP, 20 mM Na₂H³²PO₄, 25 μ g ml $^{-1}$ hexokinase, 200 mM HEPES (pH 8.25)) were added, the sample (1 ml) was deoxygenated, sealed in a small glass vial (1 cm i.d.) and immediately irradiated.

§Sonicated vesicles were prepared by sonicating (in a bath type sonicator) 2.5 mg purified soy phospholipids in 1 ml of a solution containing 1 mM tricine (pH 8.0), 3 mM MgCl₂, 0.2 M sucrose. After the suspension cleared (~15 min), 0.1 ml of the CF₀-CF₁ complex (8.0 mg protein per ml) and 0.15 ml of a solution containing 200 mM glucose, 1 mM KNO₃ was added and the suspension sonicated an additional 3 min. To this reconstituted system, 0.15 ml 10% defatted bovine serum albumin and 0.15 ml of the external buffer solution were added and samples (0.5 ml) were deoxygenated, sealed and irradiated.

incorporated into the vesicles, this result indicates multiple turnover of the enzyme. Although the relative yield of phosphorylated product (per mg protein) was greater using cholate dilution vesicles, subsequent studies were performed with sonicated vesicles to permit greater variation in both phospholipid and enzyme concentrations.

The data from several preparations of sonicated vesicles are summarised in Table 2. A reasonably good correlation was observed between the yield of phosphorylated product and the absolute quantity of enzyme added. When the CF₀-CF₁ complex was irradiated in the absence of lipid vesicles, the formation of phosphorylated product was greatly diminished, although a slight incorporation of radioactive phosphate into an organic product (~0.3 nmol per mg protein) was observed. This residual phosphorylation may result from some phospholipid

Table 2 Effect of enzyme concentration on pulse radiolysis-driven phosphorylation

Enzyme (mg ml $^{-1}$)	Lipid (mg ml $^{-1}$)	$^{32}\text{P}_i$ esterified (nmol ml $^{-1}$)	$^{32}\text{P}_i$ esterified per mg protein
0.47	2.6	3.20	6.80
0.53	1.7	1.46	2.75
1.24	2.6	3.51	2.80
1.60	13.3	5.25	3.28
1.71	26.7	4.48	2.62
1.87	26.6	6.61	3.53

Sonicated vesicles were prepared as described in Table 1, except the enzyme and lipid concentrations were varied to give the final concentrations indicated. Irradiation was with 500 pulses (50-ns pulsewidth) at 120 Hz. Dose per pulse = 1 krad.

Table 3 Substrate and enzyme requirements for pulse radiolysis-driven phosphorylation

Conditions*	$^{32}\text{P}_i$ esterified (nmol ml $^{-1}$)
Control	4.48
Heat-denatured enzyme†	0.16
ADP omitted	0.36
Enzyme omitted	0.00
Sonicated in 'external buffer'‡	2.28

*Sonicated vesicles containing 26.7 mg ml $^{-1}$ soy phospholipid and 1.71 mg ml $^{-1}$ CF₀-CF₁ complex were prepared as described in Table 1. Each sample was irradiated with 500 pulses at 120 Hz (pulsewidth, 50 ns; dose per pulse, 1 krad).

†The enzyme was heated to 55 °C for 10 min immediately before incorporation into the vesicles.

‡Sonicated vesicles were prepared as described in Table 1, except that the external buffer was added with the enzyme before the 3 min sonication step.

contamination of the CF₀-CF₁ preparation⁸, or may be related to the observation that acid denaturation of soluble CF₁, in the absence of membranes, can result in the formation of a small amount of ATP¹¹.

The data in Table 3 demonstrate that the radiolytically driven phosphorylation reaction required the CF₀-CF₁ ATP synthetase complex in active form. Furthermore, the esterification of $^{32}\text{P}_i$ occurred at significant levels only when ADP was added to the system. This strongly suggests that the initial product of the radiolytically driven reaction was in fact ATP. The interpretation is further strengthened by the finding that all of the $^{32}\text{P}_i$ -labelled product is acid stable (100 °C, 10 min, 1 M HCl), as expected for glucose-6-phosphate. These results also eliminate the possibility of a radiolytically initiated ADP- $^{32}\text{P}_i$ exchange reaction. The trace amount of $^{32}\text{P}_i$ -labelled product formed in the absence of added ADP may result from residual bound ADP added with the enzyme¹³.

Considered within the context of the chemiosmotic hypothesis⁴, the radiolytically driven phosphorylation reaction studied here appears to be reasonably efficient, although a definitive determination of the H⁺/ATP ratio cannot be made without an accurate characterisation of the structure of the vesicles used in this system. Assuming a vesicle internal radius of 100 nm, for example, the number of radiolytically generated internal protons per ATP synthesised would be approximately 800.

Surprisingly, sonication of the vesicles in the presence of high external buffer did not completely eliminate the formation of ATP in this system (Table 3). Incomplete equilibration of the external and internal buffers could account for the residual phosphorylation activity, but this does not seem likely after sonication. Alternatively, it is probable that generation of protons in ~10⁻⁸ s produces a transient large pH drop, the relaxation of which is limited only by buffer protonation. The extent to which to the initial reaction of protons with the CF₀-CF₁ complex can compete with buffer protonation reactions in the short time scale involved here could determine, at least in part, the efficiency of the overall ATP synthetic reaction, with limited dependence on the internal buffer concentration. In any event, there is evidence to indicate that the effects of internal buffers on phosphorylation efficiency are complex¹⁴.

Irradiation, in identical conditions (500 pulses, 120 Hz, 1 krad per pulse), of the solution which was used for the internal aqueous phase of the vesicle produced a long-term pH drop of ~1.2 units. Assuming a chemically inert lipid bilayer, this would allow a maximum steady state transmembrane gradient of ~1.2 pH units. A possible contribution by a radiolytically generated transmembrane electrical potential cannot be ruled out, although yields of ATP per mol of CF₁ comparable to those reported here have been measured in chloroplasts subjected to a similar small pH gradient by a dark pH jump (T. Graan and N. E. Good, personal communication).

The study of $\text{CF}_0\text{-CF}_1$ -catalysed ATP formation by the pulsed irradiation technique (Fig. 1) provides an opportunity to resolve kinetically the mechanistic processes involved in the ATP synthetase and proton pumping activities of this enzyme. Further, the ability to generate a significant transmembrane pH gradient on a nanosecond time scale should contribute to the study of a wide range of membrane processes.

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1. Ragan, I. C. & Racker, E. *J. biol. Chem.* **248**, 2563-2569 (1973).
2. Racker, E. & Kandrach, A. *J. biol. Chem.* **248**, 5841-5847 (1973).
3. Racker, E. & Stoekenius, W. *J. biol. Chem.* **249**, 662-663 (1974).
4. Mitchell, P. *Biol. Rev. Cambridge Phil. Soc.* **41**, 445-502 (1966).
5. Matheson, M. S. & Dorfman, L. M. *Pulse Radiolysis* (MIT Press, Cambridge, Massachusetts, 1969).
6. Miller, C. & Racker, E. *J. Membrane Biol.* **26**, 319-333 (1976).
7. Racker, E., Chien, T.-F. & Kandrach, A. *FEBS Lett.* **57**, 14-18 (1975).
8. Winget, G. D., Kanner, N. & Racker, E. *Biochim. biophys. Acta* **460**, 490-499 (1977).
9. Patterson, L. K. & Lilie, J. *Int. J. Radiat. phys. Chem.* **6**, 129-141 (1974).
10. Ort, M. G. & Stocken, L. A. in *Mechanisms in Radiobiology* (eds Errera, M. & Forsberg, A.) 259-331 (Academic, New York, 1961).
11. Magnusson, R. P. & McCarty, R. E. *J. biol. Chem.* **251**, 6874-6877 (1976).
12. Gould, J. M., Cather, R. & Winget, G. D. *Analyt. Biochem.* **50**, 540-548 (1972).
13. Roy, H. A. & Moudrianakis, E. N. *Proc. natn. Acad. Sci. U.S.A.* **68**, 464-468 (1971).
14. Ort, D. R., Dilley, R. A. & Good, N. E. *Biochim. biophys. Acta* **449**, 108-124 (1976).

In vivo reduction of cyclopropene by *Azotobacter vinelandii* nitrogenase

NITROGENASE from N_2 -fixing aerobes such as *Azotobacter vinelandii* is stable to air in crude extracts but on fractionation into its two protein components, Fe-Mo protein and Fe protein it manifests the O_2 sensitivity characteristic of other nitrogenases¹. This difference between the air-stable and air-labile forms of the enzyme has been ascribed to various causes²⁻⁴. More generally, the incompletely defined correlations between *in vivo* and *in vitro* nitrogenase function with respect to optimal component ratio⁵, local pH⁶, ATP stoichiometry⁷, reductant⁸, catalytic behaviour^{4,9} and other important enzymatic parameters are a source of ambiguity in biochemical studies on the cell-free enzyme. In principle, effects from changes in many of these parameters can be studied by parallel *in vivo* and *in vitro* substrate reduction and inhibition studies. In practice¹⁰, restrictive limitations are imposed by the paucity of nitrogenase substrates that are at once (1) well bound, (2) capable of diagnostically complex chemical interactions, and (3) compatible with function of the enzyme in living microorganisms. For example, among known substrates other than N_2 itself, C_2H_2 is deficient in reduction complexity¹⁰, HCN is excluded by its cytotoxicity and acrylonitrile, which displays interesting product patterns¹¹, is a poor substrate¹². Recently, cyclopropene was

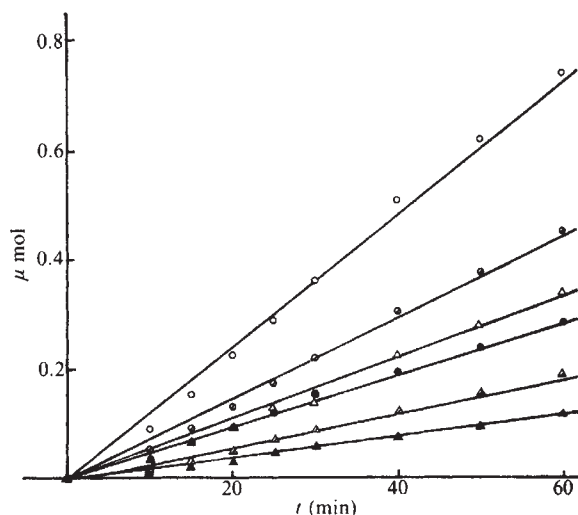


Fig. 1 CO inhibition of cyclopropene reduction by *A. vinelandii* cultured on N-free Burk's medium. The time course of formation of products was monitored by GC after addition of substrate (0.04 atm), inhibitor (as shown below), O_2 (0.1 atm) and 206 Klett-ml of log phase culture. $P_{\text{CO}} = 0.0026$ atm: propene, ●; cyclopropene, ▲. $P_{\text{CO}} = 0.0013$ atm: propene, ○; cyclopropene, △. Control (no CO): prpm, ○; cyclopropene, △.

shown to undergo ATP-dependent nitrogenase-catalysed reduction to propene and cyclopropene *in vitro*¹³. The product ratio of ~2:1 alkene:cycloalkane was found to be insensitive to purification over a specific activity range of 40-1,200 in conventional assay conditions. We report here results of further nitrogenase-cyclopropene investigations which establish that: (1) nitrogenase in living *A. vinelandii* catalyses cyclopropene reduction to the products observed *in vitro* in the same ~2:1 ratio; the K_m for formation of either product is very similar if not identical to that obtained with cell-free enzyme; (2) with purified components (specific activity up to 2,000) at optimal stoichiometry, the K_m for formation of the reduction products (propene and cyclopropene) is 0.01 atm, corresponding to a molar K_m as low as that of N_2 (and C_2H_2). These findings strengthen the case for catalytic similarity between *in vivo* and *in vitro* nitrogenase. Conversely, they substantiate the importance of cyclopropene product ratio as a chemical probe for the enzyme and as an important new criterion for nitrogenase biomimetic chemistry.

Cyclopropene (0.05 atm in Ar or He) was incubated at 30 °C and shaken in septum-stoppered 21 ml vaccine bottles with 2 ml of log phase *A. vinelandii* OP culture grown on a modified¹⁴ N-free or NH_3 -supplemented Burk's medium. Cyclopropene was prepared purified and analysed as described elsewhere^{13,15}. In aerobic assays, the gas phase also contained 1-5% O_2 (care was taken to avoid O_2 inhibition¹⁶). Respiration-dependent reduction to both propene (~2.1) and cyclopropene (~1.0) was observed with N_2 -fixing cultures; the reduction ratio remained constant throughout the linear portion of product evolution time course experiments (Table 1). Cultures grown on the NH_3 medium were devoid of cyclopropene reduction activity (Table 1). Only respiring, N_2 -fixing *Azotobacter* possess C_2H_2 reduction activity^{4,9}.

Table 1 Requirements for reduction of cyclopropene by *A. vinelandii*

Whole cell assay system	Medium	Propene activity (nm per Klett-ml min) $\times 10^3$	Cyclopropene activity (nm per Klett-ml min) $\times 10^3$	C_2H_4 activity (nm per Klett-ml min) $\times 10^3$
Complete	N-free	61	30	152
Complete	+ NH_3	<1	<1	<1
- O_2	N-free	<1	<1	<1

Values are average values for duplicate assays. See text for details of assay system.

Table 2 Comparison of kinetic parameters and product ratios for *in vivo* and *in vitro* nitrogenase reduction of cyclopropene

Enzyme state	Propene formation		Cyclopropane formation		R*	C ₂ H ₂ K _m (M) [†]	N ₂ K _m (M) [‡]
	K _m (M)	V _{max} /V _{max} (C ₂ H ₂)	K _m (M)	V _{max} /V _{max} (C ₂ H ₂)			
<i>In vivo</i>	1.3 × 10 ⁻⁴	0.38	1.4 × 10 ⁻⁴	0.20	1.9	2.2 × 10 ⁻⁴	—
<i>In vitro</i>	1.1 × 10 ⁻⁴	0.29	1.0 × 10 ⁻⁴	0.14	2.1	1.3 × 10 ⁻⁴	1 × 10 ⁻⁴

*Ratio V_{max} (propene)/V_{max} (cyclopropane). [†]This work. [‡]Ref. 1.

Inhibition of the *in vivo* cyclopropene reduction by N₂ or CO was also consistent with identification of nitrogenase as the responsible catalyst. At a partial pressure of 0.91 atm, N₂ caused a reversible decrease in the cyclopropene reduction rate of 73% at *P* (cyclopropene) = 0.02 atm. CO was highly effective in inhibiting cyclopropene reduction *in vivo* (Fig. 1). CO has been previously shown to be a potent inhibitor of N₂ and C₂H₂ reduction by nitrogenase^{1,4}. (The normal propene:cyclopropane ratio seemed slightly increased in the presence of CO.)

Lineweaver-Burk plots of propene and cyclopropane formation rate dependence *in vivo* on initial cyclopropene pressure were linear (Fig. 2a) and gave an average K_m value of 0.014 ± 0.002 atm for each product. In identical conditions, a K_m value of 0.006 atm was found for C₂H₂, in good agreement with most

literature data^{1,4}. Using a value for the Henry's law constant at 30 °C for cyclopropene of 4.6 × 10⁶ mm (C.E. McKenna and M.C. McKenna, unpublished work) and a literature value of 1.1 × 10⁶ mm for that of C₂H₂ (ref. 17), molar K_m values for the two substrates are found to be about the same (Table 2). Of other nitrogenase substrates, only N₂ has as low a K_m value^{1,4}. The ratio V_{max} (cyclopropene)/V_{max} (C₂H₂) from these experiments had an average value of 0.58.

Similar kinetic experiments were done using highly purified Av1 and Av2 recombined in a 1:2 ratio (Fig. 2b). Formation of both cyclopropene products was characterised by the same K_m value within the error of the measurement; the ratio V_{max} (cyclopropene)/V_{max} (C₂H₂) was about 0.43. Comparison of the *in vivo* and *in vitro* results (Fig. 2a,b and Table 2) reveals that the K_m values for both propene and cyclopropane formation are strikingly similar and that over a pressure range from 0.29 K_m to 1.8 K_m no variation in the propene:cyclopropane ratio is observed; the V_{max} (propene):V_{max} (cyclopropane) ratio is unchanged from the experimental ratio values.

Cyclopropene is thus comparable to N₂ and C₂H₂ in effectiveness as a nitrogenase substrate both *in vivo* and *in vitro*. As a catalyst for cyclopropene reduction, *Azotobacter* nitrogenase gives similar kinetics (K_m, V_{max}) whether in intact cells or assayed as an isolated enzyme in specified standard conditions. It is clear that its overall catalytic properties with the new substrate are not greatly affected by removal from the cell and purification; to the extent that a nitrogenase K_m value inversely reflects active site binding, this means that the relatively bulky cyclopropene molecule has equivalent access to the *in vivo* and *in vitro* enzymes.

The observations described here show the potential of cyclopropene product stoichiometry as a chemical probe for nitrogenase directly^{10,15,18} and for chemical models of the enzyme^{19,20}.

The effectiveness of cyclopropene as an *in vivo* nitrogenase substrate also suggests investigation of the substitutional relationship between cyclopropene and C₂H₂ with other C₂H₂-inhibited reductases (such as hydrogenases²¹) in N₂-fixing bacteria.

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- Winter, H. C. & Burris, R. H. *Rev. Biochem.* **45**, 209–426 (1976).
- Scherings, G., Haaker, H. & Veeger, C. *Eur. J. Biochem.* **77**, 621–630 (1977).
- Haaker, H. & Veeger, C. *Eur. J. Biochem.* **77**, 1–10 (1977).
- Burns, R. C. & Hardy, R. W. F. in *Molecular Biology, Biochemistry and Biophysics* Vol. 21 (eds Kleinzeller, A., Springer, G. F. & Wittman, H. G.) (Springer, New York, 1975).
- Shah, V. K., Davis, L. C. & Brill, W. J. *Biochim. biophys. Acta* **384**, 353–359 (1975).
- Orme-Johnson, W. H. *et al.* in *Recent Developments in Nitrogen Fixation* (eds Newton, W., Postgate, J. R. & Rodriguez-Barrueco, C.) 131–178 (Academic, New York, 1977).
- Watt, G. D., Bulen, W. A., Burns, A. & LaMont Hadfield, K. *Biochemistry* **14**, 4266–4272 (1975).
- Hageman, R. V. & Burris, R. H. *Biochemistry* **17**, 4117–4124 (1978).
- McKenna in *Molybdenum and Molybdenzymes* (ed. Coughlin, M. P.) Ch. 14 (Pergamon, Oxford, in the press).
- McKenna, C. E. *et al.* in *Nitrogen Fixation*, Vol. 1 (eds Newton, W. E. & Orme-Johnson, W. H.), University Park Press, Baltimore (in the press).
- Fuchsman, W. H. & Hardy, R. W. F. *Bioinorg. Chem.* **1**, 195–213 (1972).
- Hardy, R. W. F. & Jackson, E. K. *Fedn Proc.* **26**, 725 (1967).

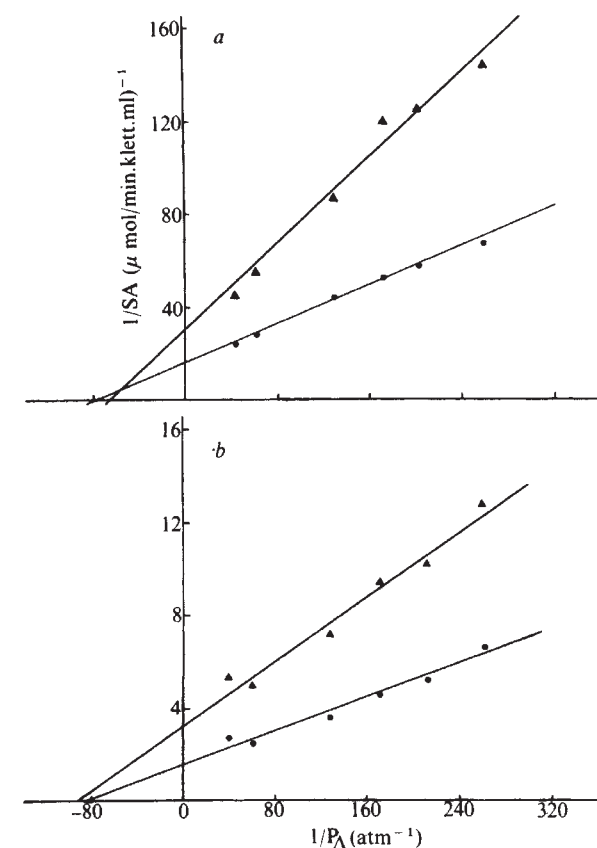


Fig. 2 a Double reciprocal (Lineweaver-Burk) plot of reduction rate dependence on cyclopropene pressure with *A. vinelandii* cultured on N-free Burk's medium. After addition of O₂ (0.1 atm) and 218 Klett-ml of log phase culture, time course plots for product formation (GC) were made at each substrate pressure and the initial rate (*V*) found from the linear portions; *V* values were divided by the product of the cell density (Klett units) and assay culture volume to obtain specific activities (SA values). The data were fitted to a linear regression curve as shown. Propene, ●; cyclopropane, ▲. **b**, Double reciprocal (Lineweaver-Burk) plot of reaction rate dependence on cyclopropene pressure with purified nitrogenase components from *A. vinelandii*. The component ratio was 1:2 Av1:Av2. Assay conditions were as described elsewhere¹³. Time course plots for product formation (GC) were made at each substrate pressure and the initial rates were found from the linear portion; *V* data were then converted to SA values (V per mg limiting protein-min). The data were fitted to a linear regression curve as shown. Propene, ●; cyclopropane, ▲.

13. McKenna, C. E., McKenna, M.-C. & Higa, M. T. *J. Am. chem. Soc.* **98**, 4657–4659 (1976).
14. Benemann, J. R., McKenna, C. E., Lie, R., Traylor, T. G. & Kamen, M. D. *Biochim. biophys. Acta* **264**, 25–38 (1972).
15. McKenna, C. E. *et al.* in *Proceedings of the International Symposium on Molybdenum Chemistry of Biological Significance* (eds Otsulca, S. & Newton, W. E.) (in the press).
16. Drozd, J. & Posgate, J. R. *J. gen. Microbiol.* **60**, 427–429 (1970).
17. Wilhelm, E. *et al. Chem. Rev.* **77**, 219–262 (1977).
18. McKenna, C. E., McKenna, M.-C. & Huang, C. W. *PNAS* (in the press).
19. McKenna, C. E., Jones, J. B., Eran, H. & Huang, C. W. *Nature* **280**, 611–612 (1979).
20. McKenna, C. E., Nakajima, T., McKenna, M.-C., & Newton, W. E. (in preparation).
21. Smith, L. A., Hill, S. & Yates, M. G. *Nature* **262**, 209–210 (1976).

Reduction of cyclopropene as criterion of active-site homology between nitrogenase and its Fe–Mo cofactor

THE nitrogenase Fe–Mo cofactor (FeMo-co)¹ is usually characterised by its unique ability to restore N₂-fixing ability to extracts of certain nitrogenase-less mutants^{1,2} and by its spectral properties, some of which can be correlated with those of the FeMo protein itself³. The role of FeMo-co in nitrogenase function is not yet clear. Recently, it was reported that uncomplemented FeMo-co catalysed BH₄[−] reduction of C₂H₂ to C₂H₄ in a CO-inhibited, non-ATP-dependent process⁴. This was taken as evidence that FeMo-co is the active site for N₂ reduction and binding in nitrogenase, although conversion of N₂ to NH₃ by cofactor-BH₄[−] mixtures was not observed⁴. C₂H₂ is readily reduced by many homogeneous and heterogeneous catalysts and can give only a single 2 e[−] reduction product, urging caution in exclusive reliance upon it as a model substrate^{5,6}. The observation that nitrogenase catalyses reduction of cyclopropene to both propene and cyclopropane in a ~2:1 ratio *in vitro*⁷ and also *in vivo*⁸ suggests a more demanding criterion⁸ for evaluation of FeMo-co catalysis, one which still avoids the difficult energetic requirements for N₂ activation and reduction. This new criterion is strengthened by the fact that cyclopropene competitively inhibits N₂ (ref. 9). We find that incubation of FeMo-co with a suitable 'apoprotein' extract yields a reconstituted FeMo-protein activity for reduction of cyclopropene to propene and cyclopropane. FeMo-co alone, however, only catalyses cyclopropane formation in the conditions described⁴ for C₂H₂ reduction. This result and other considerations suggest that active site homology between FeMo-co and nitrogenase has not yet been fully demonstrated on a function basis.

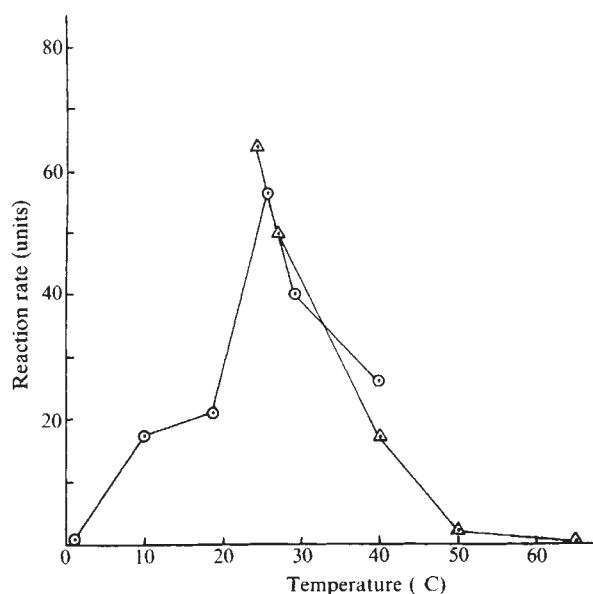


Fig. 1 Initial reaction rate-incubation temperature profile for FeMo-co-dependent BH₄[−] reduction of C₂H₂ to C₂H₄. Identical 50 μl samples of FeMo-co were assayed as described in the text. ○, experiment 1; △, experiment 2.

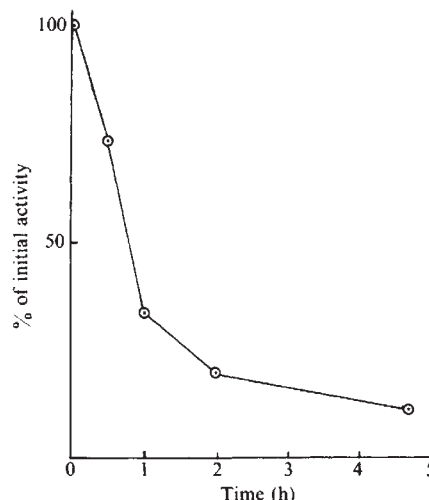


Fig. 2 Inactivation of the FeMo-co catalyst on exposure to air. 500 μl of the FeMo-co preparation was placed in an open 13 × 125 mm test tube and agitated at 150 r.p.m. in a 25 °C waterbath. At the indicated times 50 μl aliquots were removed and assayed for C₂H₂ reduction activity as described in the text.

FeMo-co was extracted from recrystallised *Azotobacter vinelandii* FeMo protein (Av1), specific activity 2,100⁹, by a procedure adapted from that of Shah and Brill¹. Typically, 140 mg Av1 in 2.8 ml of 25 mM Tris-HCl, pH 7.4 containing 250 mM NaCl was acidified by addition of 0.54 ml of 1 M citric acid at 4 °C. After 3 min, 2.4 ml of 0.5 M potassium phosphate, pH 9.0 was added dropwise with stirring and the protein precipitate was centrifuged for 7 min at 4,500 g. The pellet was extracted with 2.0 ml of dimethylformamide (DMF). The resulting suspension was clarified by centrifugation, and the pellet extracted with 2 ml of *N*-methylformamide (NMF). Both DMF and NMF were redistilled and contained 5 mM potassium phosphate (pH 8.0) and 2 mM dithionite. The NMF extract was layered on a 1 × 54 cm Sephadex G-100 column and developed in the same solvent (8 ml per h). FeMo-co elution from this column was monitored at 440 nm and was followed by 10-fold concentration under vacuum at 32 °C. All isolation and assay procedures were carried out under purified Ar. Samples were stored in liquid nitrogen and remained active for several weeks. BH₄[−]-dependent reductions were carried out in 22-ml serum vials containing (in 3 ml): 0.5 ml NMF; 80 mM sodium borate pH 9.6; 240 mM NaBH₄ and 4 mM sodium dithionite. Reaction was initiated with 50 or 100 μl FeMo-co concentrate and reduction of substrate (0.03–0.04 atm) was determined by GC. Activities (nmol substrate reduced per min) were corrected for background reduction, where necessary.

Shah and Brill⁴ reported that C₂H₂ reduction by the BH₄[−]-FeMo-co system at 25 °C was dependent on catalyst concentration but was not affected by C₂H₂ concentration (0.25–1.0 atm) nor by increasing the reaction temperature to 30 °C. No C₂H₆ was detected. Reduction activity was relatively stable to air inactivation (−16% after 18 h) at 4 °C.

Our results confirm that BH₄[−]-FeMo-co mixtures reduce C₂H₂ to C₂H₄ at an initial rate that depends on the FeMo-co concentration (determined over a range of 0 to 110 activity units). However, a dependence on the C₂H₂ concentration is observed at partial C₂H₂ pressures less than about 0.02 atm. Replacing the diluting gas (Ar) with He or H₂ (ref. 4) had no effect, and dithionite alone did not support reduction. Based on estimated Mo content, the specific C₂H₂ reduction activity of our FeMo-co preparations was 5–10% relative to homogeneous Av1 (Table 1), which compares well with the relative specific activity of 8% obtained by Shah and Brill⁴. The reaction rate increases above 0 °C to a maximum at 25–30 °C, then decreases (Fig. 1), presumably due to thermal lability of the catalytic species or to accelerated decomposition of the BH₄[−]. The maximum in the

rate profile occurring over 25–30 °C (Fig. 1) would explain the apparent invariability of the reaction rate to temperature reported previously⁴. The $t_{1/2}$ for loss of C₂H₂ reduction activity in air-exposed FeMo-co at 25 °C was nearly 1 h (Fig. 2), while the $t_{1/2}$ for loss of reconstitutive activity was less than 1 min¹. The C₂H₂ reduction activity was abolished by 0.12 atm CO or 1 mM KCN in the assay system. Besides C₂H₄, the major reduction product, we detected traces (up to 2%) of C₂H₆ (Fig. 3). A low level of background C₂H₂ reduction activity present in assay blanks did not account for this minor product (Fig. 3). Subsidiary formation of C₂H₆ is not observed in ATP-dependent reductions of C₂H₂ to C₂H₄ by either intact or reconstituted nitrogenase with dithionite as electron donor, but is typical of BH₄⁻ reductions catalysed by simple Mo-thiol complexes^{5,6,10,11}.

As shown in Table 1, cyclopropene is also reduced by the BH₄⁻-FeMo-co system, and at a rate ratio relative to C₂H₂ reduction that approximates the corresponding ratio in reconstituted (or intact) nitrogenase assayed in standard conditions. However, formation of both propene and cyclopropane was observed uniquely with intact or reconstituted FeMo protein, the cofactor alone yielding only cyclopropane. The 'apoprotein' extract from Kp 5058 used in the reconstitution experiments had no detectable catalytic activity before incubation with FeMo-co (Table 1). CO at 0.12 atm inhibited the FeMo-co cyclopropene reduction by 89%. A comparable inhibition is observed with nitrogenase at ~10⁻³ atm but the comparison is complicated by the presence of both low and high affinity CO-binding sites¹².

It seems clear *a priori* that care must be exercised in drawing analogies between the catalytic behaviour of BH₄⁻-FeMo-co⁴ and nitrogenase. ATP dependence, the reductant and the assay pH are quite different for the two systems, while the known instability of FeMo-co in anaerobic aqueous solution¹ places in question the actual species responsible for substrate reductions by BH₄⁻-FeMo-co mixtures. The striking differences in O₂-lability between the catalytic and reconstitutive FeMo-co activities may be relevant to this ambiguity, although the possibility that catalytic and complementary functions in native cofactor involve distinct and non-interactive moieties cannot be excluded. Our experiments verify that on the basis of Mo content, FeMo-co added to aqueous BH₄⁻ is a highly effective, CO-sensitive catalyst for C₂H₂ reduction⁴. C₂H₂ has been recognised as a potentially misleading model substrate for nitrogenase, especially when used alone^{5,6,9,13}. By the more stringent cyclopropene criterion, BH₄⁻-FeMo-co is not

Table 1 Comparison of nitrogenase and FeMo-co as catalysts for C₂H₂ and cyclopropene reduction

Catalyst	Relative activity (C ₂ H ₂)	Reduction rate ratio (cyclopropene/C ₂ H ₂) [‡]	Formation rate ratio (propene/cyclopropane)
Intact nitrogenase*	1.0 [†]	0.58	2§
Reconstituted nitrogenase*	—	0.56	2.5§
FeMo-co¶	0.05–0.10 #	0.40	<0.003
'Apoprotein' extract**	<0.0001	—	—

All data represent average values from two or more experiments.

* Assayed as described in ref. 7.

† Reference activity of Av1 (text) determined with 0.04 atm C₂H₂ and optimal amount of Fe protein.

‡ Equivalent pressures (0.03–0.04 atm) of both substrates. Initial rates derived from time course data were used.

§ Compare with refs. 7, 8.

|| FeMo reconstitution was performed according to ref. 1 using 5 or 200 µl FeMo-co (text) and crude extract from Kp 5058. Some variability in activity was encountered at the reconstitution step.

¶ Assayed (text) using 50 or 100 µl of stock preparation.

Ref. 4 gives a value of 0.08.

** Taken in an amount sufficient to give optimal C₂H₂ reduction activity with 500 µl FeMo-co preparation and tested under standard nitrogenase⁷ assay conditions.

functionally equivalent to nitrogenase. Interestingly, the inability of the cofactor to reduce cyclopropene to propene is shared by a number of transition metal complexes that also catalyse C₂H₂ reduction, including a Mo-thiol system¹⁴. Incubation of FeMo-co with Kp 5058 'apoprotein' extract restored propene formation. To the extent that appropriate chemical equivalence exists among (1) the FeMo-co catalyst in aqueous, alkaline BH₄⁻; (2) the spectroscopically defined FeMo-co species isolated in NMF; and (3) those Mo and Fe ions in intact nitrogenase assigned to the NMF cofactor, our findings are consistent with, but do not prove, the postulate that FeMo-co may represent only a portion of the active site responsible for binding and reducing N₂.

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- Shah, V. K. & Brill, W. J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3249–3253 (1977).
- Pienkos, P. T., Shah, V. K. & Brill, W. J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5468–5471 (1977).
- Rawlings, J. *et al. J. biol. Chem.* **253**, 1001–1004 (1978).
- Shah, V. K., Chisnell, J. R. & Brill, W. J. *Biochem. biophys. Res. Commun.* **81**, 232–236 (1978).
- Werner, D., Russell, S. A. & Evans, H. J. *Proc. natn. Acad. Sci. U.S.A.* **70**, 339–342 (1973).
- Newton, W. E., Corbin, J. L., Schneider, P. W. & Bulen, W. A. *J. Am. chem. Soc.* **93**, 268–269 (1971).
- McKenna, C. E., McKenna, M.-C. & Higa, M. T. *J. Am. chem. Soc.* **98**, 4657–4659 (1976).
- McKenna, C. E. & Huang, C. W. *Nature* **280**, 609–611 (1979).
- McKenna, C. E. *et al. in Recent Developments in Biological Nitrogen Fixation* (eds Newton, W. E. & Orme-Johnson, W.) (University Press, Baltimore, in the press).
- Schrauzer, G. N. & Schlesinger, G. J. *Am. chem. Soc.* **92**, 1808–1809 (1970).
- G. N. Schrauzer and P. A. Doemeny, *J. Am. chem. Soc.* **93**, 1608–1618 (1971).
- Orme-Johnson, W. H. *et al. in Recent Developments in Nitrogen Fixation* (eds Newton, W., Postgate, J. R. & Rodriguez-Barrueco, C.) 131–178 (Academic, New York, 1977).
- Stiefel, E. E. *in Recent Developments in Nitrogen Fixation* (eds Newton, W., Postgate, J. R. & Rodriguez-Barrueco, C.) 69–108 (Academic, New York, 1977).
- McKenna, C. E., Newton, W. E., Nakajima, T. & McKenna, M.-C. (in preparation).

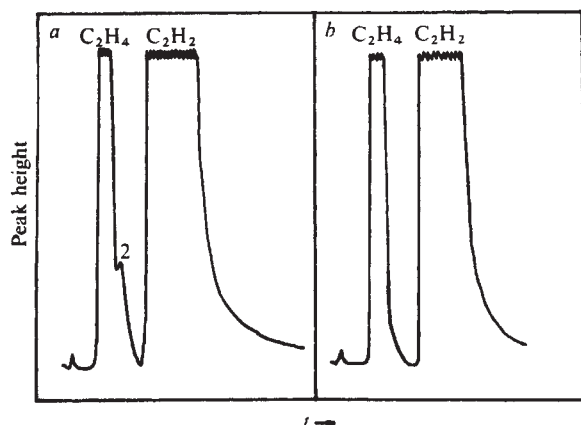


Fig. 3 Detection of C₂H₆ in FeMo-co dependent reduction of C₂H₂. The BH₄⁻ reduction assay conditions were as described in the text. GC analysis was carried out on a FID instrument equipped with a 2' Porapak N column operated at 70 °C. The He carrier gas flow rate was 30 ml min⁻¹. *a*, GC trace for 0.15 ml of the gas phase from an assay vial after 4 min incubation at 25 °C in the presence of 115 units of FeMo-co. The initial blip is due to Ar in the sample. The small shoulder labelled '2' was identified as C₂H₆. *b*, GC trace for a control vial lacking FeMo-co after 45 min incubation.

matters arising

Evolution of dominance

CHARLESWORTH¹ has noted that very harmful mutant alleles in *Drosophila* are usually almost recessive in fitness to wild type, whereas in the case of mutants which are mildly disadvantageous, dominance of the wild type is much less marked. He points out that this result agrees well with Wright's theory of dominance but argues that it does not agree with Fisher's theory, for the following reason. In the case of dominance brought about by modifiers, the rate of change in frequency of a modifier is essentially independent of the fitness of the mutant homozygote, over most of the time that dominance is evolving. Thus, given equal times for evolution of dominance, dominance should be about as marked for mildly as for seriously disadvantageous mutants, which is not in fact the case.

However, the assumption of equal times may not be justified. A mutation which is lethal or near-lethal in laboratory conditions is likely to be very harmful in almost any environment and over very long periods of time, giving ample opportunity for the evolution of dominance. A mildly disadvantageous mutation, such as a change leading to an enzyme with a slightly altered pH or temperature optimum, is less likely to be disadvantageous in all conditions or for very lengthy periods of time. Thus, on Fisher's theory we should expect evolution of dominance to be much more marked in the case of very harmful mutants. The same would be true for evolution of dominance as envisaged by Haldane. Thus, the observed differences in degree of dominance do not provide a critical test of the relative merits of the three theories.

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1. Charlesworth, B. *Nature* **278**, 848–849 (1979).

CHARLESWORTH REPLIES—The suggestion of Gale and Mackay is an interesting one, and introduces a new dimension to the theory of dominance modification. It is not obvious, however, that it invalidates my argument¹. In the first place, there is no direct evidence for their view that mutations of large effect have lower environmental variance in

their selection coefficients than mutations of small effect.

Nonetheless, even if we accept this idea, it is still not necessarily true that the conclusions of Gale and Mackay are correct. To evaluate them, we need to consider models of the modification of dominance at a locus subject to temporal fluctuations in selection coefficients. Theoretical studies^{2–4} have shown that such fluctuations generate a probability distribution of gene frequency, even in an infinite population. The intensity of selection on a modifier of dominance in a given generation can be calculated by multiplying the advantage to the heterozygote by the frequency of heterozygotes¹. The net intensity when gene frequencies and selection coefficients are fluctuating is given by the appropriate expectation over the joint distribution of gene frequency and selection coefficients. If the fluctuations in selective values are long term, the population will spend a long time in each environmental state, and so will be close to equilibrium for much of the time. When the population is in environmental state i , the intensity of selection on a modifier of effect Δh on the level of dominance of a mutant allele is thus approximated by $2u(\Delta h/h_i)$, where u is the mutation rate and h_i is the dominance coefficient in environmental state i (see ref. 1). There is thus no dependence on the strength of selection against the mutant homozygote, or on the amount of environmental change.

In the case of rapid environmental fluctuations, we need to use the gene frequency distribution at the locus subject to dominance modification^{2–4}. A complete analytical solution for this is not yet available. To reconcile the experimental findings¹ with Fisher's theory we would have to find that the net intensity of selection for dominance modification is much weaker for genes of small average homozygous effect than for genes of large effect, or for genes whose selective values are constant in time. Genes of slight average homozygous disadvantage will tend to have a gene frequency distribution with a relatively high mean³, so that the expected frequency of heterozygotes will be higher than for genes with a large effect. This will tend to counteract the smaller advantage of dominance modification in the heterozygotes, just as in the case with no fluctuations. A strong relationship between average homozygous effect, the intensity of environmental fluctuations and the advantage of

dominance modification thus seems highly unlikely. A relationship of this sort may perhaps hold, but the effect might even work in the opposite direction. A definite answer to this question can only be obtained by carrying out the relevant calculations.

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1. Charlesworth, B. *Nature* **278**, 848–849 (1979).
2. Felsenstein, J. A. *Rev. Genet.* **10**, 253–280 (1976).
3. Hartl, D. L. *Genetics* **86**, 687–696 (1977).
4. Gillespie, J. H. *Theor. Pop. Biol.* **14**, 1–45 (1978).

Haemoglobin S and *P. falciparum* malaria

PASVOL, WEATHERALL AND WILSON¹ have proposed an hypothesis to account for the protection given by haemoglobin S (HB S) against *Plasmodium falciparum* in heterozygotes for the sickle cell gene. This hypothesis depends on the argument that the last stages of a 48-h synchronous intra-erythrocytic growth cycle do not take place in the peripheral circulation, but in the deep capillary bed of some internal organ with a low oxygen tension, and that the low oxygen tension depresses invasion and development by the parasite in red cells containing HB S.

They therefore compared the rate of invasion and subsequent growth and development *in vitro* by parasites in conditions of low and high oxygen tension in red cells containing Hbs AA, AS and SS. The data given in their Table 1 do not, in our view, support the hypothesis that low oxygen tension impedes the rate of invasion and development by merozoites of *P. falciparum* in erythrocytes containing Hb S. We feel that the use of a ratio of the number of ring-form parasites per 100 red cells under low oxygen tension to that in aerobic conditions is misleading and statistically dubious, as a normal distribution could not be expected. However, one arrives at a conclusion very similar to that of the authors if one calculates the arithmetic differences for each of the pairs of measurements.

On the other hand, examination of the mean values, rather than the differences, for invasion and development in the various conditions, leads to a rather startling conclusion (our Table 1). With the exception of SS haemoglobin in aerobic conditions, all the other means are

virtually indistinguishable. However, Hb SS in aerobic conditions shows marked enhancement of invasion and development rather than, as suggested by the authors, suppression of growth in conditions of low oxygen tension. One accepts, of course, the statistical limitations of the relatively few experimental results for Hb SS.

Pasvol *et al.* also maintain that their hypothesis is supported by the data in their Tables 3 and 4. This interpretation ignores the significance of the large number of abnormal forms observed for red cells maintained in conditions of low oxygen tension (Table 3—74% for Hb AS; 20% for Hb SS—and Table 4). Furthermore, the use of a growth score from 1 to 6 for the different stages of parasite development, with abnormal forms being given an arbitrary value of 0, seems unjustifiable in the likely event that these abnormal forms represent the growth of the parasite to a stage at which no further development was possible, thus aborting the parasite life cycle and producing degenerate forms.

Table 1 Mean and s.e. for the number of ring-forms per 100 red cells after 12 h in aerobic or low oxygen conditions (data from Pasvol *et al.*¹)

	Aerobic	Low oxygen	
Haemoglobin			
AA	11.71 ± 1.48	12.32 ± 1.23	(13)
AS	11.52 ± 1.31	10.35 ± 1.34	(11)
SS	18.13 ± 2.48	11.73 ± 1.01	(4)

We have recently suggested that production of fatty acids by *Plasmodium knowlesi* and *Plasmodium berghei* may represent the molecular mechanism by which the plasmodial parasite damages red cells and produces haemolysis which may often be out of all proportion to the parasitaemia^{2,3}. Similar evidence for the involvement of fatty acids in *Plasmodium lophurae* infections has been reported⁴, and the whole question has been reviewed by Holz⁵. The fatty acids within the red cell are buffered by the haemoglobin that is present at high concentration, and Hbs AS, SS and SC are more effective than Hb AA at buffering fatty acids. This increase in buffering capacity has been rationalised at the molecular level in terms of the change in the amino acid residue at the 6β position^{3,6}, and could represent one of the mechanisms by which Hb protects against *P. falciparum* malaria.

In conclusion, we suggest that the data presented by Pasvol *et al.*¹ do not support their hypothesis concerning the mechanism for protection against *P. falciparum* malaria by sickle cell haemoglobin.

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1. Pasvol, G., Weatherall, D. J. & Wilson, R. J. M. *Nature* **274**, 701–703 (1978).
2. Laser, H., Kemp, P., Miller, N., Lander, D. & Klein, R. A. *Parasitology* **71**, 167–181 (1975).
3. Klein, R. A., Laser, H., Kemp, P., Miller, N. & Lander, D. *J. Protozool.* **24**, 39A–40A (1977).
4. Holz, G. G., Beach, D. H. & Sherman, I. W. *J. Protozool.* **24**, 566–574 (1977).
5. Holz, G. G. *Bull. Wld. Hlth. Org.* **55**, 235–248 (1977).
6. Laser, H. & Klein, R. A. *Biochem. Soc. Trans.* **5**, 292–293 (1977).

PASVOL, WEATHERALL AND WILSON
REPLY—Laser and Klein might have been less startled by the results of their analysis of our data¹ if they had considered more carefully the haematology of sickle cell anaemia; taken into account our previous work which shows that *Plasmodium falciparum* preferentially invades young, metabolically active red cells^{2,3}; and realised that the data in Table 1 of our paper deal only with parasite invasion and say nothing about development. The red cells in sickle cell anaemia have a short survival time which is reflected by a marked reticulocytosis⁴. Therefore, it is only to be expected that the degree of invasion of sickle cell anaemia (SS) cells by *P. falciparum* would be considerably greater than that of sickle cell trait (AS) or normal (AA) cells. For this reason the only valid comparisons are between cells of like ages (SS with SS, AA with AA) maintained aerobically or under reduced oxygen tensions. The data in Table 1 simply show that cells containing a significant amount of Hb S, such as SS, are invaded less actively under reduced oxygen tensions. However, we are grateful to Laser and Klein for restating that young red cell populations are preferentially invaded by *P. falciparum*.

The main emphasis of our paper was on parasite development once inside the red cells (Tables 3 and 4)¹. There is no doubt that parasites in cells containing Hb S failed to develop, but only in conditions of reduced oxygen tensions. Furthermore, sickling was not required for this to occur. Laser and Klein are quite incorrect in their assumption that the abnormal forms of the parasite maintained in low oxygen conditions represent the growth of the parasite to a stage at which no further development was possible. Rather, they represent degenerate parasites at early stages of development. This was determined both morphologically and by analysis of pigment content. Furthermore, smears prepared at an early stage in the cultures showed that these abnormal forms were already present. Even if these were entrapped mature forms, which they were not, it is difficult to understand the marked differences observed between the aerobic and low oxygen tension cultures. The growth scores used were simply a means of

condensing the data for a large number of experiments. Because the abnormal forms were mainly rings which had failed to develop, it seemed logical to rate them at less than the score of 1 designated to rings. Indeed, the results of the typical experiments in Tables 3 and 4 (ref. 1) stand without the need for growth score analysis.

Laser and Klein's hypothesis that Hb S has a greater buffering capacity than Hb A, thereby reducing fatty acid-induced haemolysis and impeding merozoite release from red cells⁵ is interesting. However, at the stage of development before merozoites are released, over 75% of the haemoglobin has been digested by the parasite⁶. Indeed, on this basis it could be argued that relatively increased sensitivity of Hb A-containing cells to lysis might result in premature death and hence offer relative protection to normal individuals with malaria! Furthermore, Laser and Klein's hypothesis is quite incompatible with the mass of epidemiological data suggesting that protection of sickle cell heterozygotes is primarily against *P. falciparum* and not against all species of malarial parasites⁷. Our hypothesis takes into account the behaviour of the mature forms of *P. falciparum* which lodge in deep tissues where the oxygen tensions are low⁸ and similar to those used in our experiments⁹. The tendency of Hb S to sickle under reduced oxygen tensions seems more rational than a subtle buffering effect of Hb S as yet not clearly defined in *P. falciparum* infections.

Thus, we believe that our findings, taken together with the essentially similar results of Friedman, who used an entirely different culture system¹⁰, indicate that the growth of *P. falciparum* is limited in cells containing Hb S exposed to reduced oxygen tensions and hence provide a reasonable explanation for the protective effect of Hb S against *P. falciparum* malaria.

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1. Pasvol, G., Weatherall, D. J. & Wilson, R. J. M. *Nature* **274**, 701–703 (1978).
2. Pasvol, G., Weatherall, D. J., Wilson, R. J. M., Smith, D. H. & Gilles, H. M. *Lancet*, **i**, 1269–1272 (1976).
3. Wilson, R. J. M., Pasvol, G. & Weatherall, D. J. *Bull. Wld. Hlth. Org.* **55**, 179–186 (1977).
4. Milner, P. F. & Charache, S. *J. clin. Invest.* **52**, 3161–3171 (1973).
5. Laser, H. & Klein, R. A. *Biochem. Soc. Trans.* **5**, 292–293 (1977).
6. Moulder, J. W. *The Biochemistry of Intracellular Parasitism*, 13–42 (University of Chicago Press, 1962).
7. Livingstone, F. B. A. *Rev. Genet.* **5**, 33–64 (1971).
8. Wilcocks, C. & Manson-Bahr, P. E. C. *Manson's Tropical Diseases* 17th edn, 939, 945 (Balliere Tindall, London, 1972).
9. Finch, C. A. & Lenfant, C. *New Engl. J. Med.* **286**, 407–415 (1962).
10. Friedman, M. J. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1994–1997 (1978).

reviews

Prediction of a global cooling

William W. Kellogg

Ice or Fire? Surviving Climatic Change. By D. S. Halacy, Jr. Pp. 212. (Harper and Row: New York and London, 1979.) \$9.95; £5.95.

THE fascinating subject of climate, and the likelihood that the world may soon witness another climatic change, has attracted the attention of several semi-popular book writers in the past few years (*Hothouse Earth*, by Howard Wilcox; *The Weather Machine*, by Nigel Calder; *The Genesis Strategy*, by Stephen H. Schneider; and *Climates of Hunger*, by Reid A. Bryson and T.S. Murray, to mention but four of the more celebrated ones). As one contemplates these very different approaches to climatic change and its many implications for mankind one is struck by the difference in tone and approach taken by the scientists actually doing climate research (Schneider and Bryson) and the science-writers who are not actively working in the field (Wilcox and Calder — and now Halacy).

It is not surprising that this difference should exist. Research scientists are trained to be critical and questioning, and are usually very cautious about jumping to conclusions too quickly (though some do, of course). This trait tends to make their scientific writings a bit dull for the average intelligent layman, although it should be quickly added that Schneider and Bryson are among the liveliest of scientist-writers.

On the other hand, a science-writer approaches his subject with the eye of the journalist, and may be on the lookout for a good story rather than a presentation of a balanced account. Halacy, in *Ice and Fire?* like Calder, has chosen to write a book whose central theme is the prediction of a global cooling as the beginning of a new ice age — perhaps occurring very quickly. Although he mentions the fact that Calder's book was criticised by many climatologists and other scientists as "irresponsible scaremongering", he has followed the same chilling path: The coming ice age, the slow growth of ice sheets over North America and Europe, the lowering of sea level as the water is trapped as ice on land, the changing patterns of precipitation, and the global scramble for new sources of food as the old ones become unfavourable are all described — almost, it would seem, with relish.

There is a strong counterpoint to this

prediction of an imminent ice age, and that is the thought that we might be able to modify or avert it if we tried hard enough. This theme is developed in Part I, which occupies more than half of the book. Halacy wrote a book called *The Weather Changers*, in 1968, and the part on weather (and climate) modification has the same title and seems to be an update on the earlier work, with an emphasis on the subject of *weather* modification — notably rainmaking. There is relatively little said about *climate* modification that would be relevant to the prospect of a coming ice age.

The book is written with an engaging style, but it gives the overall impression of a clever collage of anecdotes, theories, and facts, rather than a development of a main thesis. Furthermore, even a non-expert will notice that he has blurred his timescales cleverly (as did Nigel Calder, whom he quotes extensively), giving the impression that the advent of an ice age could occur in a matter of a decade or so — perhaps it will take a century if we are lucky, he says. This will be recognised as Calder's "snowblitz". Halacy does mention that the periodic fluctuations that have accounted for major natural climate changes are 2,500 to 100,000 yr long (although there seem to be some weaker climate changes associated with shorter periods), but this matter of timescale is never made clear in talking about the impending ice age. Few climatologists would question the likelihood of a new ice age coming *eventually* — but how many thousands of years from now?

Similarly, he does mention the idea that the burning of fossil fuels and the addition of carbon dioxide to the atmosphere is likely to cause a global warming in the next few decades, and that a "worst case" estimate calls for a warming of over 3°C by the year 2050. This, he concedes, "would play havoc with weather and climate". However, he dismisses the prospect of a warming due to human activities by saying, "There are those who reason that other pollutants stop more incoming radiation and will outbalance the CO₂ to create a cooling rather than a warming trend." This is an idea proposed some time ago by Bryson and others, but there are few left who now subscribe to it.

The expectation of a global warming in the next few decades and centuries, rather

than the onset of an ice age, was expressed most authoritatively by the more than 100 experts assembled by the World Meteorological Organization (WMO) for the World Climate Conference in Geneva in February 1979. Said the Declaration of that Conference: "We can say with some confidence that the burning of fossil fuels, deforestation, and changes of land use have increased the amount of carbon dioxide in the atmosphere . . . and it appears plausible that [this] can contribute to a gradual warming of the lower atmosphere, especially at high latitudes . . . It is possible that some effects on a regional and global scale may . . . become significant before the middle of the next century."

Thus, Halacy seems to have a rather poor batting average in his predictions, if we can judge them against current evidence; and one of his statements, presumably written a year or more ago, has already turned out to be quite wrong! "There is no evidence of a global or national [US] climate programme of significance" (p 205). Early in 1979 the US Congress passed the National Climate Program and it is now part of the law of the land; and in May 1979 the Congress of the WMO adopted the World Climate Programme. These are both official programmes being actively pursued on many levels, and the WMO's Programme in particular demonstrates the serious concern of all nations with the problems of climatic variability and climatic change.

Although *Ice or Fire?* deals with a vital set of questions, and although it contains a great deal of information recounted in a lively manner, it is seriously flawed by a combination of emphasis on subjects only remotely relevant to the main theme, and by a main theme that is itself based on a faulty premise. Perhaps if Halacy had written his book a year or two later he would have followed the lead of Wilcox and played on the prediction of a warmer Earth instead of an ice age. That too would have been a good story. □

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Geometrical aspects of general relativity

Gravitational Curvature: An Introduction to Einstein's Theory. By Theodore Frankel. Pp. 172. (Freeman: San Francisco, 1979.) £5.20.

TEXTBOOKS on general relativity fall roughly into two classes — the geometrical and the physical. As an example of the former class we have J. L. Synge's fine book *Relativity: The General Theory* (North-Holland: Amsterdam); and in the latter class there is S. Weinberg's excellent *Gravitation and Cosmology* (Wiley: New York). Theodore Frankel's book falls squarely into the geometrical division, as suggested by his title.

Gravitational Curvature starts with a short chapter on special relativity which does no more than set the scene for what is to follow. In chapters 2 and 3 Einstein's equations are approached through the principle of equivalence, the gravitational red shift and Poisson's equation. It is in the essence of the author's treatment that Poisson's rather than Laplace's equation should appear at this stage because throughout he is as much interested in the right-hand side of Einstein's equations

$$R_{ik} - \frac{1}{2} g_{ik} R = 8\pi \kappa T_{ik} \quad (1)$$

as he is in the left, an attitude which is still fairly uncommon.

It is in chapter 4 that the author declares his hand, in a section headed "A Geometric Form of Einstein's Equations", where he rewrites (1) as

$$8\pi \kappa T(\xi, \xi) = -\frac{1}{2} \epsilon_\xi R_{V^3} + \text{tr}(\mathbf{b} \wedge \mathbf{b}). \quad (2)$$

Here ξ is a unit vector at a point p in space-time V^4 , and V^3 is any hypersurface normal to ξ ; R_{V^3} is the scalar curvature of V^3 , and $\text{tr}(\mathbf{b} \wedge \mathbf{b})$ depends on the extrinsic curvature of V^3 embedded in V^4 (ϵ_ξ is ± 1). What (2) says is that however V^3 may curve at p , the left hand side (which in an older notation is $8\pi \kappa T^{ik} \xi_i \xi_k$) depends only on the normal ξ . As an application consider a perfect fluid and let V^3 be a totally geodesic space-like hypersurface (that is, one whose second fundamental form vanishes). The second term on the right of (2) disappears and we have

$$R_{V^3} = 16\pi \kappa \rho$$

ρ being the proper density, which, as the author remarks, is a particularly beautiful consequence of Einstein's equations.

The author has not derived (2) just for fun. On the contrary, he uses this form of the equations repeatedly — to derive the Schwarzschild exterior solution in chapter 5, for the general static spherical mass distribution in chapter 11, for cosmology

in chapter 12, and for other applications. In the remaining chapters are treated the perfect fluid, equations of motion, light rays, and, in some detail, electromagnetism in special and general relativity.

The treatment throughout is quite advanced. No introduction to tensor calculus or differential geometry is given, and there are no examples or exercises to help the student. Not merely is a knowledge of tensors assumed, but the reader is expected to be acquainted with differential forms, and the index-free notation as in (2) above. *Gravitational Curvature* is therefore not really a textbook. The author writes in the Preface that "ideally, the material here would be given as the third quarter of a year course

devoted to manifolds and Riemannian geometry", but it seems to me that the first two quarters of such a course would be pretty indigestible.

Who then will benefit from the book? I believe that to trained relativists it should cast a refreshing new light on the geometry of their subject. It would be nice to think that there will be some enlightenment passing in the other direction, by which I mean that differential geometers looking at the book may see how close relativity is to their subject, and even turn their attention to it for a while.

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Fludd debates

Robert Fludd: Hermetic Philosopher and Surveyor of Two Worlds. By J. Goodwin. Pp. 96. (Thames and Hudson: London, 1979.) Paperback £2.95.

CHIEFLY remembered today as the target of polemical attacks by seventeenth-century men of science like Kepler, Mersenne and Gassendi, Robert Fludd (1594–1637) was among the most famous physicians and occultists of his time. In an interesting paper, published in English translation in 1955 ('The Influence of Archetypal Ideas on the Scientific Theories of Johannes Kepler'), the physicist Wolfgang Pauli showed that the Fludd–Kepler debate could not simply be regarded as one between an occultist and a modern 'scientist'. The polemic of Mersenne and Gassendi more directly rejected Fludd's ideas in the light of the corpuscularian and mechanistic philosophies then being developed. However obscure and repellent they may now seem, some grasp of Fludd's doctrines is essential for a genuine historical understanding of an influential current which

was to be vanquished and eclipsed by the science of Galileo and Descartes.

Fludd's works are rare and have become expensive collectors' items chiefly for the beautiful engravings with which they were adorned by de Bry and Matthieu Merian in their sumptuous Frankfurt editions. A study of the engravings is essential for making it possible to penetrate Fludd's strange and fantastic cosmology. That has been made much easier by Dr Joscelyn Goodwin, a musicologist, who has brought together 120 of the plates in a well-printed and reasonably priced book.

According to Dr Goodwin in his biographical introduction, the chief value of his collection of engravings lies in reminding us "of the possibility, indeed the imminence, of a cosmic view free alike of the myopia of materialism and the absurdities of naive spiritualism". But his full bibliography will usefully guide the reader to other recent sources in which an attempt has been made to place the Fludd debates within the scientific and philosophical milieu of the seventeenth century.

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Biological foundations of sensory processing

Sensory Processing, Perception, and Behaviour. By R. B. Livingston. Pp. 106. (Raven: New York, 1978.) Paperback \$11.05.

IN his preface to this mercifully slim volume, which was aimed originally at psychiatrists, the author says his intention is "to present the main biological foundations for perception, judgement, and behavior, in an evolutionary and

developmental context.". The ground is covered in 83 pages, but the ride is uncomfortably rough.

The book is extraordinarily hard to read, and I had great difficulty in making sense of parts of it. The principal problem is the pretentious prose, which actively hinders comprehension. Here are two representative samples: "Humankind is presently in need of experiencing unprecedented perceptual, judgemental, and behavioral discontinuity, from prior to new forms of imaging"; and "similarity of views are attractive to one another, leading to greater introversion of perspectives and greater societal self-righteousness".

The other difficulty is the organisation; although the book is divided into four chapters, Behavior and Evolution, Perception, Sensory Processing, and Sensation, the order of topics in the last three is arbitrary, and seems quite confused. I often failed to grasp the thread of an argument simply because the discussion was so disconnected.

Underneath these deficiencies, I found the work interesting, principally because its broad coverage introduced me to some unfamiliar observations and ideas. However, I valued the book less for the author's synthesis of general principles than for the provision of references and pointers to other reading.

The chapter on Behavior and Evolution sets the tone for the rest of the monograph. Here Livingstone makes the point (not new to psychologists) that the idea of tight connections between sensory stimulus and behavioural response is an impoverished view of what controls behaviour. He then goes on to develop the argument that man's flexible and complex behaviour depends upon his development of a large brain, which is only possible if infants are immature at birth, which in turn means that most of man's skills have to be learned. So man becomes the

flexible animal. These arguments are plausible but, in common with many made in the book, are not developed thoroughly enough to permit careful examination.

The chapter on Perception introduces the idea that our capacity to structure our sensory input depends on our having, in our brains, adequate models of the world. Here Livingstone touches upon ideas that have been influential in perceptual psychology for nearly forty years, but he does not acknowledge this work. His discussion also neglects many worthwhile and exciting developments in 'Cognitive Psychology', and the insights into perceptual processing that have been obtained from work on the recognition of objects by computer programs. The result is a naively optimistic view of the ease with which psychological phenomena can be related to physiological events in the brain.

The chapter on Sensory Processing contains an odd mixture of topics that are not well integrated. Lateral Inhibition is included, as might be expected, but so is a summary of the psychology of perpetual development, which I found surprising. I had expected to see some discussion of broad principles that are common to the operation of the different sensory systems

—for example, Müller's doctrine of specific nerve energies and its modern counterpart, the single unit doctrine—but nothing of the sort is mentioned. Moreover, there is no discussion of the sensory codes used for the transmission of information. There are interesting sections on feedback and feedforward control mechanisms, and on the centrifugal control of afferents, but again the author fails to place these in any larger context.

The final chapter, on Sensation, is a good deal more specific than the others, and the major part of it is devoted to a straightforward review of the physiology and anatomy of the different receptor systems and their associated pathways. I was again disappointed that the author did not bring out common principles in their operation.

Livingston's aim was to provide a coherent view of how modern biology can help us better understand some old problems in philosophy and psychology. He falls short of his goal.

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Victorian medicine

The Scientific Revolution in Victorian Medicine. By A. J. Youngson. Pp. 237. (Croom Helm: London, 1979.) £9.95.

THE bookshop browser whose eye is caught by this title might well turn to the preface to learn about the aims of the book and to the concluding chapter to find a summary of the content of the book. Impressed, he might take it home, where a more leisurely inspection would reveal a strangely unrelated text sandwiched between the preface and conclusion.

We are wisely advised in the preface to avoid the trap of thinking that all who resisted new ideas were merely dull or obscurantist, but the sandwich filling falls into this very trap: "pre-scientific" medical men suffered from "slovenly thinking"; they "did not know enough medicine" (the author means *our* medicine); Sir Henry Holland was "not altogether foolish" in following Celsus' advice about the need for cheerfulness in the sickroom — that of six prime ministers and two queens, in Sir Henry's case — but in doing so he "seriously underestimates the importance, or at least the potential importance, of scientific and technical [viz, twentieth century] medicine".

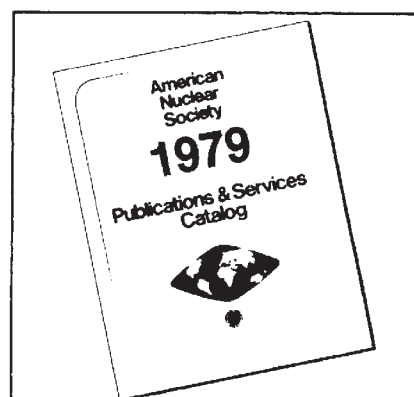
The modern observer of the early

nineteenth-century hospital might well be "struck with the utmost astonishment" if he was a modern doctor, but, I suggest, if he were a modern historian he would understand the absence of precautions when the threat was not known; he would have doubted whether some speculations of the time were "sensible" simply because they were later proved to be right; and he would certainly have disagreed with the notion that the renaissance idea of the generation of the barnacle goose was a *denial of modern biology*.

The concluding chapter attempts a formal analysis of the reasons for resistance against new ideas, and a number of interesting points are raised. These should have been tied in more closely with the previous chapters, where the examples that illustrate the general statements are comparatively standard accounts of the introduction of anaesthesia, antisepsis and related topics. These accounts differ from others largely in the author's dependence on the *Lancet*. To have these accounts together in one volume is convenient, at least, and the historical style might well prove attractive to those with an interest in the history of their profession.

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Plant protection

The Plant Protection Discipline: Problems and Possible Developmental Strategies. By W. H. Sil, Jr. Pp. 190. (Wiley: Chichester, UK, and New York, 1979.) £17.75.

THE title of this book is misleading, although the subtitle does imply that it is concerned not with a factual account of the subject, but with a discussion of certain aspects. It is essentially a series of essays which consider the problems caused by pests, diseases and weeds in the major agricultural crops of the world, the ways in which research, development and advice are used to bring them under control, and the conflicting interests involved.

The problems illustrated are almost exclusively American, although there is some reference to the work in developing countries of international agencies such as FAO and the Ford and Rockefeller Foundations. There is an excess of quotations which are so numerous and conflicting that it is difficult to discern the author's own views. Indeed he apologises for them in his introduction: "This is not a scientific treatise. Rather it is a compendium and summary of the thoughts of the leading proponents or opponents of this new discipline. Consequently, there are many quotations, far more than most scientists, including the author, might prefer."

The book is intended for "government officials, administrators and leaders in the various sub-disciplines." It argues the case for an interdisciplinary approach to the increasingly serious problem of crop losses from pests and diseases. This subject is certainly one of urgency and the reader is given a comprehensive account of a situation which has arisen chiefly from increasing concern about the pollution of the environment by pesticides and an increase in the incidence of resistance and tolerance of pests and diseases to chemical control. (A recent report from the UN Environment Programme shows that the number of insect and mite species which have developed tolerant strains increased from 182 in 1965 to 364 in 1977.) The crop losses caused by pests, diseases and weeds are serious and worldwide, and the sophisticated systems of monoculture in the developed countries are as dependent on effective pests and disease control as are the more primitive systems of the developing countries which are seeking to exploit the improved varieties bred specifically for the Green Revolution.

To meet this challenge Dr Sil maintains that there is a need for a new profession to educate 'general practitioners' of plant protection. He complains with some reason and factual support that agriculture is a low prestige subject, especially in developing countries and that applied research in agriculture is less prestigious than pure research. He distinguishes differences in attitudes between entomologists and plant pathologists, a distinction which is not nearly so significant in Europe as it seems to be in America. There is brief reference to legislation governing the use of pesticides and schemes for certification of pesticides and those who apply them. There is a lengthier consideration of the need for cooperative efforts between the various disciplines and agencies involved and at times, the author becomes obsessed with the technical jargon of 'crop protection', 'plant protection', 'pest management', 'Biological control', 'integrated pest

control' and so on, so that he loses sight of his main theme in the minutiae.

The remaining chapters of the book are devoted to a consideration of possible organisational strategies as they relate to collaboration between official research and advisory organisations and the chemical industry. The emphasis throughout is on the integration of education, research and advice.

There is little new in this treatment but the book serves a useful purpose in bringing together many topics that are of current worldwide concern. Interesting though the discussion may be, however, the readership of the book is likely to be very limited. It is hardly a book of reference because both the situation and attitudes are changing all the time; and it is expensive for what it is.

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HLA and H-2

HLA and H-2: Basic Immunogenetics, Biology and Clinical Relevance. By H. Festenstein and P. Demant. Pp. 215. (Edward Arnold: London, 1978.) Paperback £9.

TRANSPLANTATION immunology has evolved in an attempt to understand, and prevent, rejection of foreign grafts. Although we may have been getting closer to this goal, the impact of this research has not been restricted to that old dream of restorative medicine but has contributed fundamentally to a better understanding of vertebrate genetics and immunological phenomena in general, ranging from tumour immunology and serology to such complex phenomena as immune response regulation and the fascinating linkage between susceptibility to disease and major transplantation antigens.

This booklet on HLA and H-2 covers this wide area in a short and accessible way. It is intended for the many clinicians and immunologists who do not have the time, or courage, to study the encyclopaedic treatises on H-2 by J. Klein or by Snell, Dausset and Nathenson. Taking transplantation immunology as "art pour art" the book is teaching a great deal: How to perform HLA typing economically and reliably, how to inbreed mice, the grand rules of serology and genetic analysis, how to cope with statistics of gene linkage, that complement factors and other MHC products may have interesting things in common, some ideas about the mysterious MIs locus or the L antigen in mice, a simple method to use sperm as Lad typing cells, and a great number of most helpful tables on various antigen systems, frequencies of antigenic

specificities, cross-reactivities, the newest rules in terminology and designation of specificities. Although the booklet has a small format, it is really an extended review, and it has been difficult to treat the immense abundance of facts and speculations on HLA and H-2 even in 200 pages. That the intended brevity has, except for MHC-coded *Ir* genes, left out some of the recent insights into the biological role of major histocompatibility gene products in defining T cell effector function, restriction specificity and responsiveness is (and my view is obviously biased) a pity. Some explanation of the probably real biological functions of major transplantation antigens might make it easier for the reader and student to get a feeling for the linkage between cell-mediated immunity against intracellular parasites (for example, viruses) and graft rejection (with all consequences for immunosuppressive therapy), for polymorphism (really the basis for transplantation reactions), and associations of disease susceptibilities and major histocompatibility antigens. However, people concerned with the practical consequences of major transplantation antigens in graft rejection are interested in the hard facts, methods of analysis and their correct evaluation of everyday experiments in the clinic or the laboratory, rather than in philosophical discussions on evolution and function of major transplantation antigens. For these practical aspects this booklet is of great value to students and practitioners in transplantation immunology and will serve as excellent and handy *vademecum*.

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● Paul Colinvaux's *Why Big Fierce Animals are Rare* (Princeton University Press: New Jersey; for review, see *Nature* 273, 787; 1978) will be published in the UK in November by George Allen and Unwin (London) at £6.50.